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## ERRATUM •

Vol. XII, 2, Plate XIV, for (1) read (3), for (3) read (1).

## THE GENETICS OF VARIEGATION IN A FERN.

By IRMA ANDERSSON,

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(With Plates I and II.)

*Adiantum cuneatum* is a common greenhouse fern belonging to the Leptosporangiate family Polypodiaceae. The variegated form here studied has been cultivated for many years at the John Innes Horticultural Institution, and in many Botanical Gardens. The original plants were multiplied by division and new variegated individuals had been also raised from spores. In the cultures from spores, a few white sporophytes were observed among the variegated ones. The prothallia were considered to be all green, and were so described by Bateson who alluded to them in the Croonian Lecture (*Proc. Roy. Soc. B*, xci. 1920, p. 368). Since white ferns had been seen to arise from the prothallia as well as variegated ferns, and the prothallia had appeared to be all green without variegation, the inference was drawn that genetic segregation had occurred in the haploid tissue of the gametophytes. It was subsequently discovered that the prothallia, though on the soil they look all green, nevertheless often exhibit pale stripes when examined as transparencies. A correction to this effect was therefore published (*Nature*, vii. 1921, p. 233) by Bateson. This made a closer examination of the phenomena desirable, and the work was kindly handed over to me by Mr Bateson in 1921.

The result of this fuller study has been to show that *all* the prothallia which are green and viable sooner or later develop pale stripes, and that *all* the green ferns which arise from them and are viable, eventually develop the characteristic variegation.

As shown in Fig. 1 the variegation of the sporophyte takes the form of patches, which to the naked eye seem white, and are wedge-shaped owing to the mode of cell-division at the growing point, or have the form of white stripes in the green tissue. Occasionally a whole pinna is white and more or less undeveloped. The amount of white patches differs slightly among the sporophytes, and there is sometimes also a difference in this respect between fronds or pinnae of the same plant,

TABLE I.

*Showing Proportion of Green : Pale Green by Germination of the Spores.*

Ref. No.	No. of Sporophyte used	Colour of frond used	No. of culture	No. of record	Result			Ratio green : pale
					Spores giving proth.		Spores ungerminated	
					Green	Pale		
1	I	variegated	A	a	1058	2164	15	1 : 2.045
2	"	"	B	a	133	299	1	1 : 2.248
3	"	much variegated	A	a	63	130	11	1 : 2.063
4	"	"	"	b	63	113	26	1 : 1.793
5	"	slightly variegated	A	a	75	98	0	1 : 1.306
6	"	"	"	b	94	141	0	1 : 1.500
7	"	"	B	a	60	96	0	1 : 1.600
8	II	variegated	A	a	283	580	30	1 : 2.040
9	"	"	"	b	281	536	27	1 : 1.907
10	"	"	B	a	83	215	4	1 : 2.590
11	"	"	"	b	88	193	2	1 : 2.192
12	III	variegated	A	a	77	195	7	1 : 2.532
13	"	"	"	b	86	201	4	1 : 2.337
14	"	much variegated	A	a	129	304	3	1 : 2.356
15	"	"	"	b	121	284	4	1 : 2.347
16	"	"	B	a	93	204	4	1 : 2.193
17	"	slightly variegated	A	a	78	142	2	1 : 1.820
18	"	"	B	a	151	305	2	1 : 2.013
19	"	"	"	b	110	204	6	1 : 1.854
20	"	"	C	a	81	185	0	1 : 2.283
21	IV	variegated	A	a	90	260	0	1 : 2.888
22	"	"	"	b	108	294	0	1 : 2.722
23	"	"	B	a	73	205	1	1 : 2.808
24	V	variegated	A	a	139	284	2	1 : 2.410
25	"	"	"	b	76	138	1	1 : 1.815
26	"	"	"	c	91	189	2	1 : 2.076
27	"	"	B	a	80	133	4	1 : 1.662
28	"	"	"	b	91	185	2	1 : 2.032
29	"	"	"	c	65	139	1	1 : 2.138
30	"	"	C	a	48	102	0	1 : 2.129
31	"	"	"	b	85	159	0	1 : 1.870
32	VII	variegated	A	a	104	234	3	1 : 2.250
33	"	"	"	b	91	201	1	1 : 2.208
34	X	variegated	"	a	130	286	2	1 : 2.200
35	"	"	"	b	100	256	3	1 : 2.560
36	"	"	"	c	121	270	2	1 : 2.247

Thus it has been found, for instance, that an otherwise green frond of a variegated plant (see Expts. 81-102, Table II) had only a single white stripe on one pinna. Owing to the fact, however, that fronds of the sporophyte are more than one cell-layer thick, observations in this respect cannot be so accurate as on prothallia. Sporangia on white patches do not develop properly, and when a sorus stands on the border between the white cell-layers of a patch and the green tissue, the white sector of it contains only pale undeveloped sporangia empty of spores, whereas sporangia in the green sector of the same sorus are perfectly normal and contain the usual amount of spores. The normal spores have the same size and as good germination as those of the green

form of *Adiantum cuneatum*. Of sporophytes from which spores were taken for experiments referred to in this paper, Nos. I, IV and X are several years old, and were produced by division from the previous stock. Nos. II and III, likewise old plants, were raised from spores, and Nos. V and VII had been raised from spores  $1\frac{1}{2}$  years before these experiments began.

Fronds or separate pinnae of these sporophytes were gathered when the sporangia were ripe, thoroughly washed under running water to remove foreign spores possibly sticking to them, and put into petri dishes to dry and shed the spores. The latter were afterwards gathered (with a sterile scalpel) and sown on the culture media. Various soil mixtures, as well as various illuminations, were tried to ascertain if external conditions have an influence on the variegation. Strong light is deleterious, but otherwise no influence was found. Some soil mixtures are more suitable for the rapid growth and development of prothallia than others.

To be able to observe the gametophyte generation more exactly throughout its life-time without disturbing the growth, it was necessary to make cultures on an artificial and transparent medium on which the prothallia could develop normally. Such a medium was found in agar with Knop's solution. After adding the white of an egg and carefully filtering in the autoclave this medium is quite transparent. Spores sown under sterile conditions on a film of this medium in petri dishes, when kept at the requisite moisture by adding Knop's solution when necessary, germinate within ten days, grow to prothallia, and send up sporophytes, which can be kept growing for a considerable time.

Control cultures of the green form of *Adiantum cuneatum* were made in all experiments.

On germination of the spores two kinds of prothallia were recognisable, some with normal dark green chloroplasts, and others having small, pale green chloroplasts. The difference of the chloroplasts in these two kinds of prothallia is apparent from the moment of germination. The prothallia containing pale green chloroplasts soon stop growing, and were not observed to reach a bigger size than ten cells, usually not more than six. Meanwhile the plastids become more and more irregular in shape, and the green colour seems to vanish. There is never a doubt to which class a prothallium belongs, since no intermediates can be found. The numerical records in Table I were taken by the aid of the cylinder marker, designed by De la Rue (*Bot. Gaz.* xx. 1920), and the prothallia on a space of 2.5 sq. cm. were scored, and this in some instances

TABLE II.

*Showing Proportion of Green : Pale Green Prothallia by Germination of Spores from separate Sporangia.*

1	2	3	4	5	6	7	8	9	10
							Result		
Ref. No.	No. of sporophyte used	Colour of frond used	Colour of pinna used	Description of sorus and indusium	No. of sorus on same pinna	No. of sporangium	Spores giving prothalli		Ungermi- nated spores or prothalli not found
							Green	Pale	
37	I	variegated	variegated	by side of	A	a	13	34	17
38	"	"	"	white patch,	"	b	5	27	32
39	"	"	"	green	"	c	7	24	23
40	"	"	"	"	"	d	5	32	27
41	"	slightly variegated	green	green	A	a	2	26	36
42	"	"	"	"	A	a	19	37	8
43	"	"	"	"	"	b	15	28	21
44	"	"	"	"	"	c	16	37	11
45	"	"	"	"	"	d	32	25	7
46	"	"	"	"	"	e	7	49	8
47	"	"	"	"	"	f	10	43	11
48	"	"	"	"	"	g	13	35	16
49	"	"	"	"	"	h	10	38	16
50	"	"	"	"	"	i	13	33	18
51	"	"	"	"	"	k	11	48	5
52	II	variegated	variegated	half green,	A	a	19	21	24
53	"	"	"	half white,	"	b	7	21	36
54	"	"	"	green part	"	c	15	41	8
55	"	"	"	used	"	d	13	19	32
56	"	"	"	"	"	e	19	33	12
57	III	"	"	between two	A	a	28	33	3
58	"	"	"	white stripes,	B	a	19	39	6
59	"	"	"	green	"	b	2	55	7
60	"	"	"	"	"	c	3	55	6
61	"	"	"	"	"	d	9	54	1
62	"	"	"	"	"	e	29	32	3
63	"	"	"	"	"	f	0	61	3
64	"	"	"	half white,	A	a	10	51	3
65	"	"	(sorus on green	green	"	b	10	42	12
66	"	"	half used)	"	"	c	4	51	9
67	"	"	green	green	A	a	6	58	0
68	"	"	"	"	"	b	2	62	0
69	"	"	"	"	"	c	5	29	30
70	"	"	"	"	"	d	21	43	0
71	"	"	variegated	by the side of	A	a	17	43	4
72	"	"	"	white patch,	"	b	12	44	8
73	"	"	"	green	"	c	14	47	3
74	V	slightly variegated	green	green	A	a	25	36	3
75	"	"	"	"	"	b	22	31	11
76	"	"	"	"	"	c	20	39	5
77	"	"	"	"	"	d	10	52	2
78	"	"	"	"	"	e	14	40	10
79	"	"	"	"	"	f	14	43	7
80	"	"	"	"	"	g	12	38	14
81	"	green except for this	variegated	green	A	a	28	36	0
82	"	variegated pinna	"	"	"	b	7	44	13
83	"	used here	"	"	"	c	16	37	11
84	"	"	"	"	"	d	15	46	3
85	"	"	"	"	"	e	14	42	8

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TABLE II—continued.

Ref. No.	No. of sporophyte used	Colour of frond used	Colour of pinna used	Description of sorus and indusium	No. of sorus on same pinna	No. of Sporangium	Result		Ungerminated spores or prothalli not found
							Spores giving prothalli		
							Green	Pale	
86	V	green <i>except</i> for the	green	green	A	a	21	42	1
87	"	variegated pinna	"	"	"	b	22	30	12
88	"	used in 81-85	"	"	"	c	19	42	3
89	"	"	"	"	B	a	21	43	0
90	"	"	"	"	"	b	15	32	17
91	"	"	"	"	"	c	18	41	5
92	"	"	"	"	"	d	9	43	2
93	"	"	"	"	"	e	26	38	0
94	"	"	"	"	"	f	28	29	7
95	"	"	"	"	"	g	8	40	16
96	"	"	"	"	C	a	17	32	15
97	"	"	"	"	"	b	24	36	4
98	"	"	"	"	"	c	23	41	0
99	"	"	"	"	"	d	18	32	14
100	"	"	"	"	"	e	14	48	2
101	"	"	"	"	"	f	19	39	6
102	"	"	"	"	"	g	27	31	6
103	"	variegated	variegated	green	A	a	9	27	36
104	"	"	"	"	"	b	13	33	19
105	"	"	"	"	"	c	3	11	50
106	"	"	"	between two	A	a	32	13	19
107	"	"	"	white stripes,	"	b	8	28	28
108	"	"	"	green	"	c	22	14	28
109	"	"	green	green	A	a	11	7	46
110	"	"	"	"	"	b	9	9	46
111	X	"	green	green	A	a	16	44	4
112	"	"	"	"	"	b	12	42	10
113	"	"	"	"	"	c	23	41	0
114	"	"	"	"	"	d	28	28	8
115	"	"	"	"	"	e	23	35	6

repeated in other places of a culture. The numerical relation of dark to pale green gametophytes by germination of the spores is shown in Table I.

The table shows that the ratio of green:pale green ranges from 1:1306 (No. 5) to 1:2888 (No. 21). The same table indicates that the amount of variegation on different fronds does not at all affect the proportion of the two chlorophyll types which arise on germination of the spores contained in their sporangia.

A certain regularity exists in the fact, as seen in the table, that the number of dark green never exceeds the number of pale green.

That this segregation is a normal phenomenon and that the ratio of green to white cannot be altered by an increase, diminution, or loss of any of the constituents of the culture medium, was clearly shown by trial on different media. Neither could the pale prothallia or white sporophytes be kept growing, nor the plastid colour be altered by the

addition of any of the ingredients of Knop's solution, or by transplantation to a medium in which the proportion of these constituents was varied, or the strength of the agar increased. The small white prothallia are also present in soil cultures kept under the best conditions, and they are absent from control cultures of the green form of *Adiantum cuneatum*.

The cultures thus far described were grown from spores taken from various sporangia collectively. It was nevertheless desirable to ascertain how the various kinds of spores might be distributed in individual sporangia. The cultivation of spores from single sporangia offered attractive possibilities, inasmuch as in the Leptosporangiate ferns the sporangium is an outgrowth of a single epidermal cell. This protrudes and after some divisions forms the sporangium with tapetum and spore mother-cells, where reduction eventually takes place at spore-formation. Every sporangium was found to contain the 64 spores predicated by the regular divisions in the archesporium, but owing to the technical difficulties of transferring the spores from the glass-cell, in which the sporangium was kept to shed its spores, to the culture medium, some of them were lost as indicated in col. 10 of Table II. As shown in this table there is no evidence that separate sporangia, arising from sporophytic cells of different constitution, give a ratio in any way corresponding to what might be expected from the appearance of the sporophytic tissue surrounding the original cell. On the contrary every sporophytic cell (except the white ones in the patches) seems able to give both kinds of prothallia.

The variability of the ratio green to the pale green (for convenience also called white) as shown in Table II is astonishingly wide and ranges from 2 green : 62 white as in No. 68, to 25 green : 32 white in No. 45. The amount of green never exceeds 32, and when there is an excess it is always of the whites. To this point I will return in the discussion.

The spores are known to occur in two shapes, called tetrahedral and bilateral. They may also occasionally vary in size, but on experiment (Table III) it appeared that these distinctions were in no way related to the characters of their contents: for either of the two classes of prothallia may come from any class of spore.

TABLE III.

No. of Sporophyte	Spores big, tetrahedral give		Spores small, tetrahedral give		Spores bilateral give	
	White	Green	White	Green	White	Green
III	27	10	5	4	60	25
V	45	22	44	28	14	5



All the prothallia, recorded and spoken of above as green to indicate their appearance immediately after germination of the spores, sooner or later acquire one or more pale-green patches, the largest number observed being seven. They are thus variegated like the sporophyte. If the sporophytic and gametophytic generation is each taken as a whole, we have thus white and variegated gametophytes in the proportions of about 2 : 1, and sporophytes of two kinds, namely variegated and white. To the probable explanation of the occurrence of white sporophytes I will return in the discussion. To ascertain if all growing prothallia are variegated and that there are no green ones, a hundred very young prothallia were transplanted, one in each pot, and kept damp by watering from below, the pots being covered with glass. In this way they attain a considerable size before sending up the sporophyte and reveal their nature. Subsequently another hundred were so raised. All these sooner or later became variegated.

As the prothallia for a long time grow with a two-sided apical cell, most of the pale patches originate at this growing point. Wedge-shaped pale-green patches or stripes result after further cell-divisions. This sudden change of the chlorophyll happens, however, also at divisions elsewhere in the prothallium, either along the margin (especially in older prothallia), in which case the stripes will continue in continuity with the edge, or in a cell at any point inside the margin in which case a patch, surrounded on all sides by green cells, is formed. Occasionally the whole growing point is occupied by a patch, after which no more green cells are formed at this growing point.

The patches, like those of the sporophyte, look white to the naked eye and can, when they have attained a bigger size, easily be found. When viewed, however, under the microscope it is found that the pigment of the chloroplasts in cells of the patch is only a dull grey-green, or a shade paler than that of the chloroplasts of the surrounding cells, which can be described as vivid dark-green. Under higher magnification it would perhaps sometimes be difficult to tell where the border between dark and pale cells comes, if one had not at the same time the size of the plastids to go by. This is specially the case in the initial cells of a patch. Chloroplasts in the prothallia of the green *A. cuneatum* as well as in the normal green cells of these variegated prothallia are dark-green, of regular form, and mostly cover the surface of the turgescient cell. Dividing stages are easily recognizable, and throughout these investigations when measurements of chloroplasts were made, cells and chloroplasts in a state of division were avoided. This is, however, difficult in the growing-point.

When the patches consist of many cells, for instance about 300, and there are thus several cells between the dark-green celled borders, it is found that the pale cells of the patch along the border immediately adjoining the dark-green border-cells contain plastids, the colour of which is more intense than that of the plastids in the middle of the stripe. This clearly indicates that plastids in cells bordering the green normal ones are to a certain degree fed or influenced by the latter. When the stripes or patches are narrow the difference in colour between the middle and the edge is either imperceptible or at least not so pronounced. The hesitation as to which is the original cell of a patch (as mentioned above) is due to the same cause. Moreover the plastids of the cells of the patch are smaller than those of the normally green tissue. Though well-formed and apparently healthy, their size is reduced probably in correlation with the difference in colour. This distinction in size also is greatest in the central cells of a patch, which are like those of the prothallia which germinate white.

Both normal dark-green and pale-green chloroplasts contain starch, but in the latter the amount is very small.

Measurements of plastids were undertaken to make out how much the sudden change in the original cell of a patch did affect the size of plastids; whether this change or the effect of it in the original cells in different cases or different prothallia was the same; and whether the change was big enough in the supposed original cell to leave no doubt about where the change had taken place. It was *a priori* possible that plastids of various sizes (and colour) might be present within the same cell anywhere in a prothallium, and especially in the cells originating a patch. Moreover there might be indications of a gradual decrease in size of plastids in cells leading up to that one in which the actual change took place.

It was also of interest to see if the plastids, once they had been affected by the change in the initial cell, would diminish at subsequent divisions according to any measurable rate. The measurements were undertaken on perfectly intact prothallia in water, and were only continued so long as the cells were normally turgescant, and the plastids retained their normal shape. The more rounded shape of a plastid not in full vigour is easily recognizable. In each cell as many plastids as possible were measured. Each chloroplast was measured twice, the longest and shortest diameters at right angles to each other. (Leitz objective No. 6 and micrometer ocular No. 12.) These two numbers were multiplied together and averaged. In Table IV the average sizes

are shown (1) of the dark-green plastids in cells lying next to the basal end of the stripe; (2) the pale-green plastids in the initial cell of the patch; (3) the second size expressed as a percentage of the first. In prothallium VII, given in the Table, the patch No. 2 was entirely surrounded by green tissue. In prothallium III, patch No. 2, and in prothallium V, patch No. 1 were still in connexion with the growing-point when measured.

TABLE IV.

No. of prothallium	No. of patch	Average size of plastids in green collocated cells*	Average size of plastids in pale initial cells expressed in sq. $\mu$	Size of pale plastids as percentage of that of the green
III	1	38.18	21.50	56.5
III	2	35.82	11.68	32.6
IV	1	32.0	21.09	65.9
V	1	28.82	16.60	54.8
VI	1	36.56	20.18	55.2
VI	2	38.16	20.40	53.4
VI	3	39.05	25.86	66.0
VII	1	37.70	18.98	52.1
VII	2	44.14	27.20	61.5

As shown in Table IV the difference is considerable. The plastids are suddenly reduced to about half their size. The condition in this respect is similar for different patches and prothallia. Through very extensive measuring it was proved that the average size of the plastids in the green cells lying next to the basal cells of the patch did not vary more than the size of plastids elsewhere in the prothallium. Never has a cell containing plastids of the two or more categories been found, whether among those adjacent to the stripe or elsewhere. Neither could there be found a decrease in size of plastids on passing up or down the chains of green cells. In the patches when the reduction in size has been once brought about, no perceptible further change appears to take place. The size of plastids in cells from the initial cell upwards (along the border in older and anywhere in younger patches) to the edge of the prothallium does not diminish more than do the green normal plastids in control-rows, that is they are kept the same. The chloroplasts along the middle of big patches I have previously described, and it is clear that neither of these phenomena can be attributed to a sorting out of different kinds of plastids, or of their primordia, by cell-divisions. The change affecting the chloroplasts is sudden and definite. Probably the change affecting the colour is primary and that of the size secondary, because though in the green normal parts of prothallia young chloroplasts as small as the

\* The green cells chosen for these measurements were those lying in close proximity with the basal end of the pale stripes.

pale green can be found, the colour is nevertheless the dark green and the pale plastids never attain the full size.

The patches may appear at any time in the life of the prothallia, and do not arise in any period with special frequency.

The nature of the segregation visible in the tissues of the gametophyte constitutes a special problem. The green and the white areas in the prothallia presumably differ in their genetic capacities, and inasmuch as the prothallium is a haploid structure a genetic segregation has almost certainly here occurred after the reduction division, in which the characters of the spores were determined. Unfortunately direct proof of the genetic properties of the white areas cannot be obtained. Archegonia are rarely borne on the white stripes when, of course, their wall is white. Antheridia are scarce. When present they stand at the base of the prothallium and have never been seen to be included in a white stripe. When albino ferns have been seen to arise they have always been on green areas.

Mention should also be made of the observation several times repeated that in spite of the fact that the cotyledon was white, fronds containing green chlorophyll have been produced from the growing-point of the young sporophyte; and conversely, in association with a variegated cotyledon, a white, non-viable sporophyte has arisen. This suggests that the white sporophytes result from a segregation at an early division of the embryo, though they might also be the product of the union of two gametes factorially without green.

Investigation is not so far advanced as to justify definite conclusions concerning the cause or nature of the process resulting in this defect of the chlorophyll.

Since all the prothallia which survive eventually become striped, and all the ferns which live sooner or later become variegated, there are therefore in this respect only two classes of both gametophytes and sporophytes, viz. (1) the variegated, and (2) those which are white. So far as the variegated ferns are concerned, they might be supposed to be heterozygotes between a green-bearing and a white-bearing gamete, but this account is not applicable to the prothallia since they are haploid. The defect in the plastids may safely be assumed to be of the same nature, both in the ferns and in the prothallia, and all that can be declared is that this defect occurs sporadically, and is sooner or later inevitable in both. Once it has happened, the tissue affected is incapable of producing normal green plastids again. I have calculated that an original white cell arises during the life of the prothallia once in about 10,000 cell-divisions.

With this the frequency of the occurrence of white cells in the sporangia should be contrasted.

On spore-formation spores with defective plastids are produced very numerous, and at most only 32, viz. 50 % can carry the power to form normal plastids, a total rarely attained. This scarcely looks like any familiar type of segregation. Another indication of some unusual process is to be found in the four (out of nine) examples in which the outcome of all 64 spores was recorded, with the result that *odd* numbers of the two types were produced, viz.

No.	Green	Pale
70	21	43
89	21	43
98	23	41
113	23	41

According to the received account from the originally single central cell of the sporangium 16 cells are formed by 4 successive divisions; and on the 5th division (into 32 cells) reduction occurs, the last division into 64 cells being an equational-division. Without "non-disjunction" or some other error of cell-division, 2 classes containing odd numbers cannot ensue from such a process.

Up to the present time only a preliminary investigation of the cytology has been made and upon this I hope to report later.

Further, the distinction between the green and the white may be rather a matter of plastid-inheritance than of nuclear or cytoplasmic constitution, and in that respect we are probably right in regarding the distinction between diploid and haploid as out of account.

I am much obliged to Mr Bateson for assistance with this work, and to Miss D. M. Cayley for many suggestions.

## DESCRIPTION OF PLATES.

### PLATE I.

Fig. 1. *Adiantum cuneatum* var. *variegata*. Upper surface of frond.

Fig. 2. Pinnae with sori: natural size.

Fig. 3. Prothallium with one pale stripe. ( $\times 12$ .)

Fig. 4. Prothallium on germination with pale chloroplasts. ( $\times$  about 200.)

Fig. 5. Prothallium on germination with normal green chloroplasts. ( $\times$  about 200.)

Fig. 6. Drawing of part of prothallium showing pale cells and green cells in collocation with them. ( $\times 330$ -350.)

The pale cells numbered 1-8 are presumably the initial cells of the stripe.

### PLATE II.

Fig. 7. Microphotograph of part of a prothallium showing a white stripe and the adjacent cells in detail.

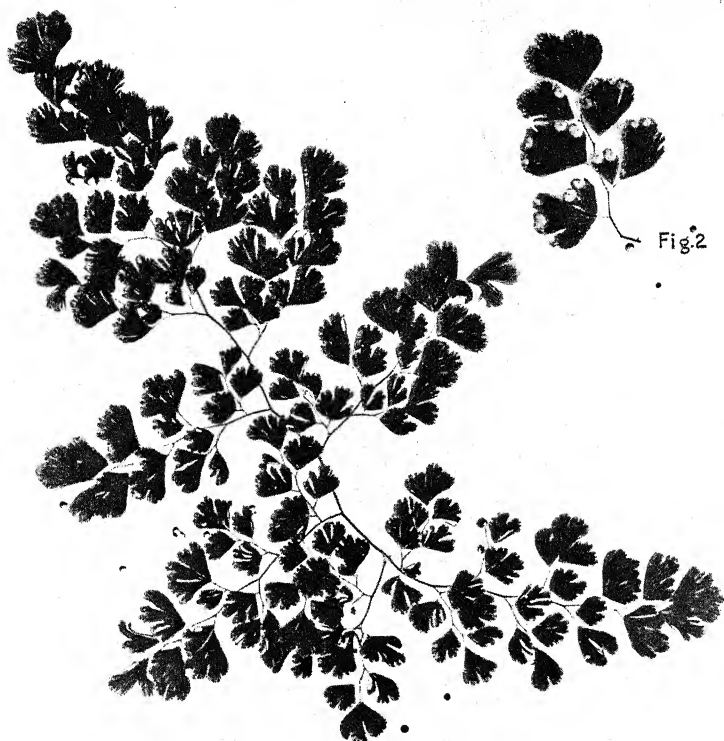


Fig. 1.



Fig. 3.

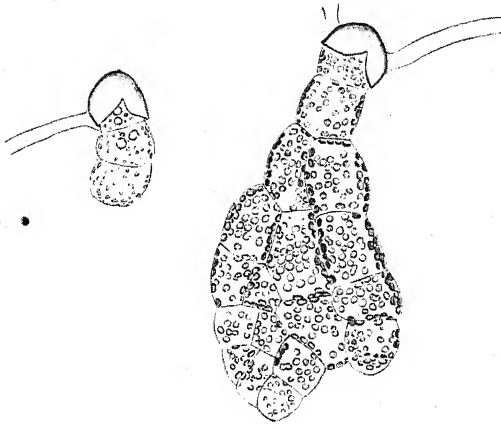


Fig. 4.

Fig. 5.

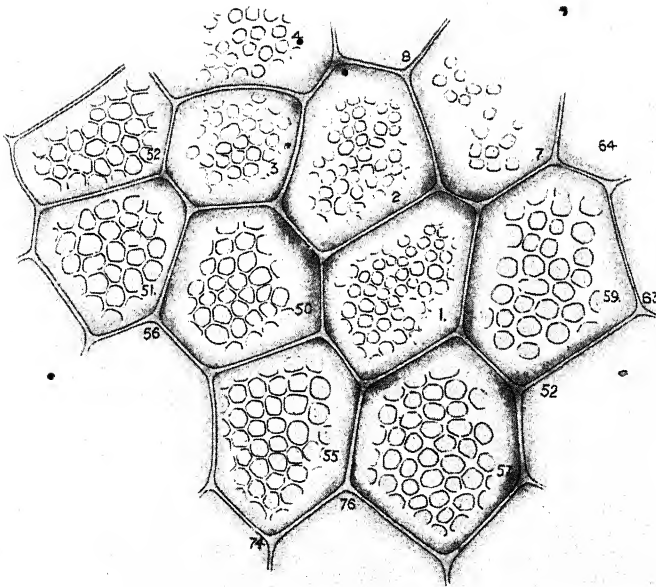


Fig. 6.





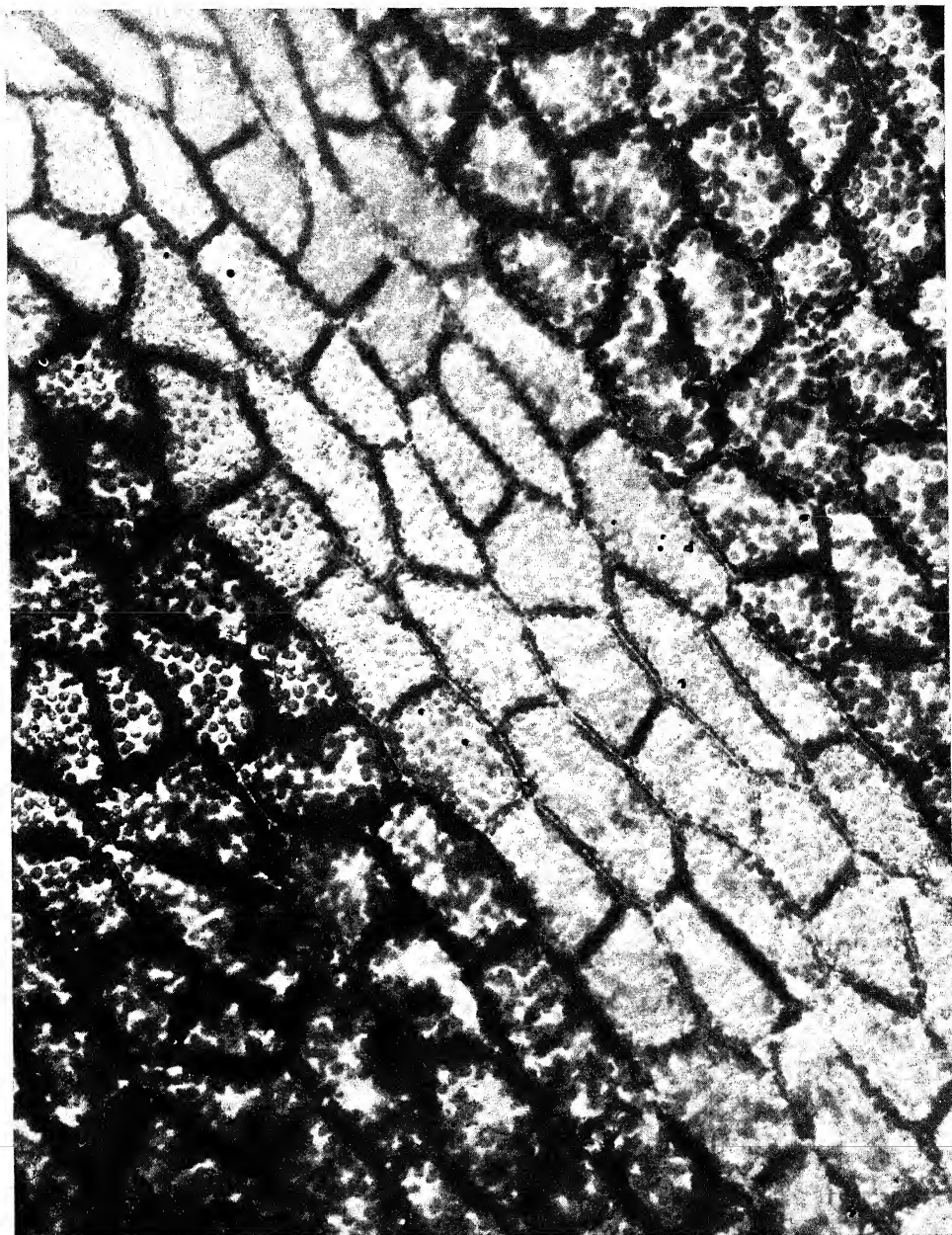


Fig. 7.



# A PECULIAR TYPE OF VARIABILITY IN PLANTS.

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(With 24 Text-figures.)

THE study of size inheritance, with the exception of nanism and gigantism, which will not be considered here, has never been in the same satisfactory condition as many other phases of genetics. This applies particularly to such characters as flower-size or length of petals, where there is repetition of parts in the same individual, but it also applies to studies of size, weight and stature in animals and man. Johannsen (1903), in his studies of size in pure lines of beans, demonstrated the necessity for recognizing a sharp line of distinction between inherited and non-inherited quantitative variations, *i.e.*, between mutations and fluctuations, in experimental practice; and it is now almost universally agreed that such a sharp distinction must be drawn in experimental work on heredity and variation. Geneticists have not, however, generally recognized that partial inheritance may constitute a third category of phenomena (see Gates, 1914, p. 560) bridging to some extent the gap between the other two. It is at any rate becoming evident that the conception of fluctuations as environmentally or nutritionally produced modifications falling into a symmetrical Galtonian curve or half curve of variability and devoid of any element of inheritance will no longer serve to characterize *all* variations which are not mutational in character.

East (1913, 1916a, 1916c) and Emerson and Hayes in particular, in a number of joint papers (see literature list) have been active in showing that size inheritance can at any rate be stated in terms of the Mendelian notation. This view has been based on the fact that the  $F_1$  is relatively uniform and intermediate in size (though it may be nearer the larger parent owing to heterosis) while the  $F_2$  shows a more extended range of variability. In some cases the original parental size may be recovered or even exceeded in the  $F_2$  or later generations; in other crosses this does not happen. When the parental sizes are not recovered it is usually

assumed that this is because insufficient numbers have been grown. It is agreed on all hands that there is an absence of dominance in connection with ordinary size factors, although dominance is well known to occur in crossing many types of dwarfs. All factors controlling size are therefore not alike in their hereditary behaviour.

But the single fact of increased variability in  $F_2$  is not in itself a proof of the presence of several Mendelian size-factors, however reasonable this interpretation may appear. The absence of dominance and the continual presence of fluctuations obscure the results and hence no instance of the inheritance of multiple Mendelian size-factors can as yet be regarded as proven, in the sense in which ordinary behaviour of multiple Mendelian factors, with either complete dominance or separate recognition of the heterozygote, and clear segregation, is proven. The studies of size inheritance in plants have dealt particularly with such characters as length of cob in maize, and length of corolla and size of leaf in tobacco. The relative uniformity and intermediacy of the  $F_1$  generation in crosses between parent stocks which differ in any of these size characters is generally indicated; also the greatly increased range of variability in  $F_2$ . And without further analysis of the results it is reasonable to interpret them as has been done, and to conclude (East 1916a) that the "evidence tends to justify the use of plural segregating factors in interpreting size inheritance." But a further difficulty, which has not been appreciated by geneticists, has arisen in connection with the study of the size inheritance of repeated parts in the same individual. In the investigation of such subjects as length of cob in maize or size of flower in tobacco, it has frequently been assumed that one specimen from a plant would be representative of the whole plant. No attempt has been made to test the range of variation on the individual, and where visible variations in size have occurred on a plant it has usually been tacitly put down to "fluctuation." The remarkable range of petal-size found on some of the hybrid *Oenothera* plants, shows that such an assumption cannot safely be made.

It can, then, be shown that a type of segregation is occurring in some crosses involving size differences, which is not in conformity with the Mendelian scheme. There are facts in relation to the inheritance of flower-size which do not fall in with this conception. The present writer (Gates, 1917) has described in certain crosses between large- and small-flowered *Oenotheras* a uniform  $F_1$  followed by an  $F_2$  and  $F_3$  in which a great variety of petal-lengths may occur on the same plant, and even different lengths of petal in the same flower. Obviously this cannot be

explained in terms of Mendelian segregation, although in the same hybrids such segregation is actually taking place as regards the red pigment character of the buds. This fact serves to contrast vividly these two types of hereditary behaviour. At the same time it serves to set before us a type of variability which does not fall within the definition of fluctuation, and in which there is an element of inheritance. This type of variability is obviously entitled to recognition as a distinct category of variation, for it differs in fundamental respects from both fluctuations and mutations; from the latter in that the inheritance (or at any rate its expression) is strikingly variable on the same individual; from the former in that (1) the variability in petal-length in the flowers of an individual seldom if ever conforms to the Galtonian curve, and (2) because this condition of variability has arisen as the result of crossing and the peculiar manner of inheritance or expression of the differences which entered into the cross. It is probable that various cases which have passed as instances of Galtonian fluctuation curves are really of this nature.

The present paper is in part a continuation of my former paper (Gates, 1917) in which were presented the results in  $F_1$ ,  $F_2$ , and  $F_3$  of reciprocal crosses between *Oenothera biennis* and *Oe. rubricalyx*. In the present account I wish to deal with the  $F_1$  generation from this cross, and also to analyze more fully the data already published for the  $F_2$  and  $F_3$  generations. It is first necessary to recapitulate briefly some of the main results contained in my former contribution on this subject, and as that paper was written during the war when my earlier records were unavailable, it will be possible now to add certain data regarding the  $F_1$  generation.

The original reciprocal crosses were made in 1912 at the John Innes Horticultural Institution, Merton, and the  $F_1$  generations grown in 1913 at the Agricultural Experiment Station, Rothamsted, not at Merton as stated in the above paper. Only three plants matured in the  $F_1$  of *Oe. biennis*  $\times$  *rubricalyx*, and two plants in the reciprocal. Their general characters were as before described, showing the red pigmentation of *rubricalyx*, but otherwise a general intermediate condition as regards foliage, although nearer the *rubricalyx* parent. The flower-size was intermediate and appears to have been quite uniform. Measurements from one plant (Sept. 5) showed length of petals 30 mm., length of hypanthium 33 mm., length of ovary 16 mm., length of bud cone 25 mm., length of sepal tips 6 mm., diameter of hypanthium 3 mm., diameter of bud cone at base 8.5 mm. No other measurements were

made, but for reasons to be given later, it is probable that the  $F_1$  was uniform in flower-size.

In 1915 one  $F_2$  family was grown at the Missouri Botanical Gardens, St Louis, from each of the  $F_1$  families. No certain differences could be determined between the reciprocal crosses. Both  $F_2$  families resembled the *rubricalyx* parent except in having smaller flowers which were self-pollinated in the bud. Striking variations in size of flower were soon observed, and the measurements made are recorded (Gates, 1917) in Table II, p. 241. The first ten lines of that table refer to *Oe. rubricalyx*  $\times$  *biennis* and the remainder to the reciprocal cross. It was also recorded that the petals of the same flower often varied several mm. in length. These results, in which different sizes of flowers were obtained blooming simultaneously on the same plant, and even different lengths of petals in the same flower, were so striking that the  $F_2$  was grown in the experimental grounds of the University of California, at Berkeley, California, in 1916, on a larger scale. In the  $F_2$  there was (1) a range of petal-length for the whole population from 14–32 mm., (2) a marked difference in the average or mean length of petals for each plant, (3) sometimes a conspicuous difference in length of petals on the same flower. The range of petal-length for an individual plant was never great, the greatest recorded in  $F_2$  being 16–24 mm. There were thus in  $F_2$  (a) indications of segregation in flower-size between different plants, (b) irregular "segregation" between different flowers on an  $F_2$  plant in certain cases. All these results were accentuated in the  $F_3$  records, probably owing to the larger numbers grown.

A single  $F_3$  culture (16.72) was grown from *Oe. rubricalyx*  $\times$  *biennis*. In the parent plant of this culture, the four flowers which were measured (on Aug. 18, 1915) ranged in size from 18 to 23 mm. in length. If measurements had been taken throughout the blooming season this range would probably have been increased, but there does not appear to have been marked segregation in petal-size on this plant. In the  $F_3$  culture from this plant, the results of extensive measurements have already been recorded (Gates, 1917, Table IV) in such a way that the arrangement of the flowers on each plant and the petals in each flower can be determined. The extreme range of petal-length was from 7–39 mm., or from less than half the size of petals in *Oe. biennis* to nearly the full size of petals in *Oe. rubricalyx*; and this notwithstanding the narrow range of size observed in the parent plant.

In Table I of this paper these results are grouped so as to show the frequency distribution of length of petal for each  $F_3$  plant. It will be

TABLE I.  
Frequency distribution of Petal Length in *P. Plants* (*Oe. rubricalyx* × *biennis*).

Culture 72/16

Plant	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
34	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
41	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
42	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
43	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
44	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
46	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
47	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
48	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
49	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
50	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
51	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
52	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
53	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Totals	4	3	13	20	13	20	17	20	38	25	25	30	26	45	32	38	55	68	94	68	87	84	60	139	62	84	57	54	38	20	6	1	2

=1344

observed from this table that while there are marked differences in range of flower-size between different plants, the range seldom groups itself about a single mode as in fluctuating variability, but there are often two or more modes with a haphazard distribution of intermediate lengths. Plants No. II. 6 and II. 36, however, show narrow ranges of variation, with modes on 32 and 34-35 respectively, and an approximation to a very steep Galtonian curve. The range of variation in other plants may be great or small. It may form a continuous series or there may be more or less marked gaps in the series of flower sizes. Clearly we are not dealing with fluctuating variations, nor yet with Mendelian segregation. That definite size-units are involved is perhaps possible, but the data furnish no clear and unequivocal support for such a conclusion. In plant II. 7 the petal sizes are in two groups, one running from 14 to 20 mm., and the other from 24 to 33 mm., with a gap between them. Again, in plant II. 19 we have one series running from 11 to 20 mm. with a break, and another series ranging from 30 to 36 mm., with another break. The wide gap from 20 to 30 mm., is very difficult to account for on the theory of the presence of several cumulative size-factors. Indeed this conception cannot be applied in the ordinary way to a phenomenon which is essentially one of somatic segregation within the individual and cannot depend upon segregation in germ cells.

Let us consider in this connection the remarkable cases of plants II. 21, and II. 37. In the former the range of petal-length is from 7 to 31 mm. with two conspicuous gaps in the series. The petal-lengths range themselves in three groups respectively 7-11 mm., 15-18 mm. and 22-31 mm. There would appear to be a tendency, as in other plants, to "split off" smaller petals with mean length about 10 and 15 mm. respectively. Similarly in II. 37 the range is from 9 to 30 mm., with wide gaps between 30, 22 and 15 mm., and another clear mode at 11 mm. These plants then show clear evidence of "segregation." Plant II. 47 shows a considerable range from 7 to 23 mm. but all its flowers were relatively small, while plant II. 45 shows modes at 13, 27-28, and 34 mm. In nine of its flowers the petals are grouped around 34 mm. as a mode, in two around 27 mm., and in one flower around 13 mm. Is it reasonable to assume that in the development of the primordia of the smaller flowers, say two and five size-factors respectively were lost? We doubt very much if any analytical advantage can be gained by taking such a view of the matter. As will be seen from Table IV of my former paper, plant II. 19 is chiefly remarkable for the widely different lengths of petal in the same flower. In an *Oenothera* flower the petals are normally of



uniform length within two or three millimetres. Here they range in a single flower from 19 to 34 mm. or from 13 to 20 mm., and the total range is from 11 to 36 mm., which is considerably greater than the whole range between the original parents. Between many wild species of *Oenothera* there are characteristic and constant differences of only a few mm. in length of petal, while the range of variation within a pure species does not normally exceed a few mm.

From an examination and tabulation of all the modal points in Table I, it appears that there is no marked tendency for them to group themselves at particular points when the psychological tendency to mass the measurements on the round numbers 15, 20, 25, 30 is eliminated<sup>1</sup>. The totals in Table I are regrouped in Table IA for this purpose.

TABLE I A.

Petal lengths	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Totals	...	4	3	13	13	20	16	17	20	38	25	27	30	26	45	32	38
									27	28	28			34	34	35	
Petal lengths	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Totals	...	55	68	94	68	87	84	60	139	62	84	57	54	38	20	6	1
			76	77	77			87	87	87							

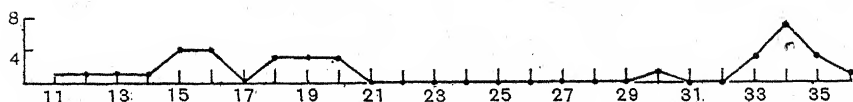
No tendency to aggregate on 10 appears, probably because the measurements in this part of the table were nearly all intercalated singly among longer petal measurements. With the numbers around 15, 20, 25 and 30 thus redistributed, certain points come out clearly. (1) There is practically a steady progression in frequency of petal lengths from the minimum size recorded up to 32 mm., which is approximately the size of the  $F_1$  hybrid flowers. Between 32 and 40 mm. (the size of the large parent) there is a steep and steady fall in the curve of frequency. This is shown in Fig. 8, which is drawn from the corrected values in Table IA. (2) It will be seen from the corrected values that there are more or less marked gaps in frequency at several points, namely between 13 and 14 mm., also 22-23 mm., 23-24 mm., and 32-33 mm. While various possible explanations of these gaps might be suggested, it is not believed that they furnish any evidence of the segregation of definite fixed units.

<sup>1</sup> This tendency no doubt arises from the fact that when the actual reading is, for example, intermediate between 30 and 31 mm. it will more often be read 30 than 31. We believe, however, that the necessity for making this correction only applies to the  $F_3$  measurements, as the necessity for greater accuracy was recognized in the  $F_4$  measurements.

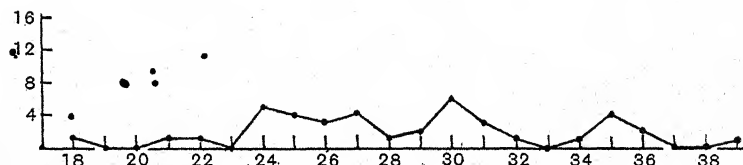
Figs. 1-7 are curves showing the character of the variability in certain selected plants from the  $F_3$  culture 16.72, and illustrating the variety of types of curve met with. They are similar to those obtained later in the  $F_4$  generation, but they show generally a significantly wider variation. Fig. 8 shows the curve of variability for the population treated as a whole when the above-mentioned corrections have been made.

In dealing with these results, the conception of fixed units of any kind being segregated or inherited as such, appears to break down

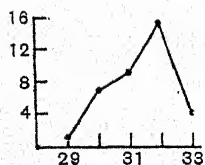
II. 19



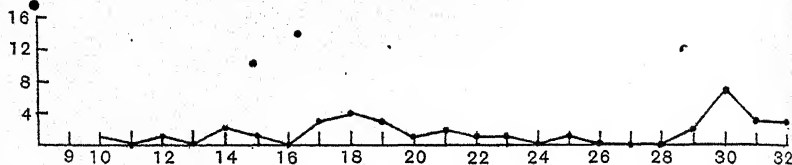
II. 9



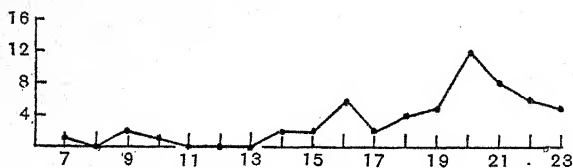
II. 6



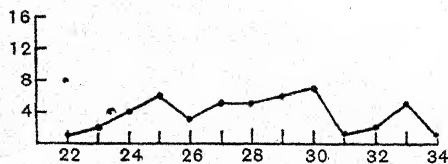
II. 12



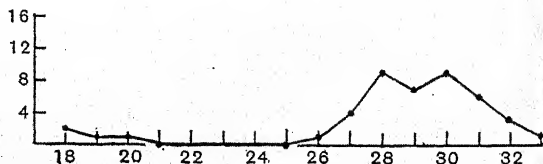
II. 47



II. 10



II. 14



Figs. 1-7. Frequency Distribution for Individual Plants of  $F_3$ . *Oe. rubricalyx*  $\times$  *viennis*.  
From Culture 72/16.

completely. Essential as this conception is for the study and analysis of Mendelian inheritance, it cannot be usefully applied here so far as we can see. It will, therefore, make for clarity of thought if we recognize that we are dealing here with something which is not only not Mendelian in its nature, but something to which different conceptions of an inheritance process will probably have to be applied. That there is an

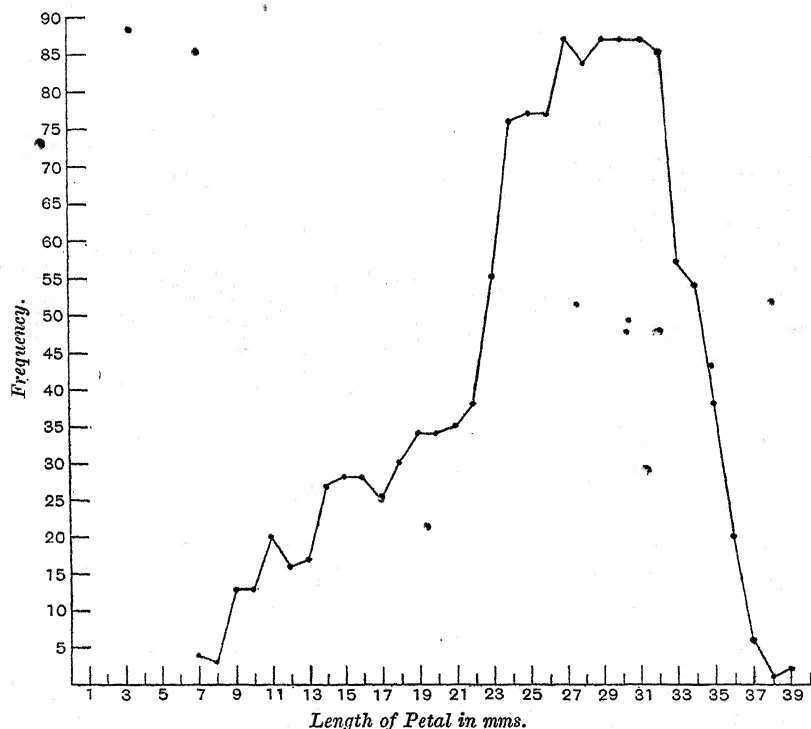


Fig. 8. Culture 72/16.

element of inheritance in this phenomenon of variability or somatic segregation, cannot of course be doubted, because it only occurs in the offspring of crosses between large-flowered and small-flowered forms. Nevertheless, such segregation or gaps in the variation series as occur may apparently occur equally at any point, if correction is made for the source of error in measurement already pointed out. This conclusion is reached after a careful analysis of the data in Table I from this point of view. Study of all the data leads to the conclusion that while *fixed* units can hardly be present, yet definite units subject to a sliding scale of quantitative variations are probably involved. It is very doubtful

if definite factors affecting size alone are ever present in these cases of cumulative size inheritance. It is far more likely that sizes are determined by physiological factors which chiefly determine other differences but incidentally control the size-production of various organs. The validity of any attempt to analyse the inheritance of flower-size in terms merely of fixed size-factors is therefore open to grave doubt. It is merely a makeshift method of studying size until the real nature of the genetic factors involved is better understood. The same applies to other studies of size and weight inheritance.

Turning now to the  $F_4$  generation, measurements were obtained on a large scale from four  $F_4$  families, numbers 1, 10, 11, and 53, grown in 1920 at the Royal Botanic Gardens, Regents Park, London. In addition several other  $F_4$  families were grown. The four families from which extensive measurements were made were all derived from the self-pollination of different plants in the  $F_3$  family No. 16. 72. Culture 20. 1 came from plant number II. 14, and 20. 53 from plant number II. 6. The records of variation in petal-size in these particular plants may be seen from Table IV of my former paper, and Table I of the present one. Also the range of variation in seven of those plants is plotted in Figs. 1-7. It will be observed that while II. 6 showed a narrow range of fluctuations almost symmetrically grouped around 32 mm. as a mode but exhibiting negative skewness, No. II. 14 exhibited a much wider range, with one early flower markedly smaller than the others which ranged from 26 to 33 mm. in length.

The  $F_4$  cultures 20. 10 and 20. 11 were derived from different flowers of a single plant (II. 50) in the  $F_3$  culture 16. 74 from the reciprocal cross, *Oe. biennis*  $\times$  *rubricalyx*. The behaviour was exactly the same in both crosses, and so only a few measurements were made of the latter cross in  $F_3$ . The following records were kept, however, of the plant II. 50. The sixth flower from the base was the first to bloom, on June 27, 1916. This flower had petals of maximum size (42 mm.). The five flowers below were still in bud. The lowest flower on the stem was the smallest, being as small as in *Oe. biennis*. This single plant then exhibited flowers as different in size as the two original parents. The length of bud cone in the five unopened flowers was at that time as follows: lowest bud 20 mm., 2nd, 23 mm., 3rd, 27 mm., 4th, 27 mm., 5th, 30 mm., 6th, 30 mm. The first and fifth flowers were enclosed in bags and left to develop their seeds, since they were self-pollinated in the bud. From these flowers were derived respectively the  $F_4$  cultures 20. 11 and 20. 10. The intention was to compare their offspring, to determine if this large difference in

flower-size would result in any difference in the next generation. The only other petal measurements made on this plant were of three flowers on July 14th, with the following results:

36	34	36
35	35	35
35	35	36
35	35	36

In Table II, the frequency distributions for petal-length of eight plants in culture 20.10 and 33 plants in culture 20.11 are recorded. The total number of plants to bloom was much larger, but lack of time prevented more measurements being taken. It does not appear that further measurements would have significantly altered the results. Culture 20.10 contained 140 plants, of which 134 had the red buds of *rubricalyx* and six the green buds of *rubrinervis*. This character showed as heretofore sharp Mendelian segregation, giving a ratio of 22:1. This probably indicates the presence of two factors for red, as in previous generations (see Gates, 1914, 1915). In culture 20.11 there were 68 plants, 64 having red buds, 2 green and 2 doubtful, since they were mutants and did not come into bloom. One of the latter, a narrow-leaved dwarf, resembled the form *jaculatrix* de Vries, the other remained a rosette. The ratio 64R:2r in this family is probably comparable with 134R:6r in the other family from this plant.

From Table II it will be seen that in culture 20.10 the mode for different plants ranged from 27-30 mm. (probably non-significant), the mode for the whole population falling on 30 mm., with strong negative skewness. For culture 20.11 the mode ranges from 25-30 mm. in different plants, with frequently more than one peak to the curve and a good deal of scattering. The mode for the whole culture falls on 27 mm., with positive skewness as regards frequency and negative as regards range. Whether this difference in the mode for petal length from the two flowers is significant of a difference in heredity I am not prepared to say in the absence of more extensive data. But it is worth noting that the first 8 plants in culture 20.11 taken together give essentially the same curve as the whole culture, with the mode in the same place and a similar frequency distribution of the other classes. It is, therefore, quite probable that the difference between these two cultures is a significant one. This probability is increased by the fact that the means (see Table V) differ in the same way, being respectively 27.3 for culture 20.11 and 29 for culture 20.10. It is also worth noting that the range of variation is essentially the same in both cultures, i.e. 19-33 mm.

TABLE II.

Frequency distribution of Petal Length in two  $F_4$  Families from Plant II. 50  
in culture 16. 74 Oe. biennis  $\times$  rubricalyx.

Culture 10/20		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
I. 1	—	—	—	—	—	—	—	—	—	—	3	7	3	7	—	—	—
I. 2	—	—	—	—	—	—	—	—	2	1	11	9	1	8	—	—	—
I. 3	—	1	—	—	—	1	—	5	5	6	10	5	5	8	2	—	—
I. 4	—	—	—	—	—	—	—	—	1	3	4	5	4	11	4	—	—
II. 2	—	—	—	—	—	—	—	—	—	—	—	1	11	23	1	—	—
II. 3	—	—	—	—	—	—	—	—	—	—	2	6	6	14	4	—	—
II. 4	—	—	—	—	—	—	—	—	—	—	1	—	4	8	6	4	1
II. 5	—	—	—	—	—	—	—	—	—	—	—	—	1	12	5	2	—
Totals ...	1	—	—	—	1	—	—	5	8	10	31	33	35	91	22	6	1 = 244
Culture 11/20		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
I. 1	—	—	—	—	—	—	—	4	16	3	1	—	—	—	—	—	—
I. 2	—	—	—	—	—	2	—	2	10	4	2	—	—	—	—	—	—
I. 3	—	—	—	—	—	—	1	5	1	7	15	5	2	—	—	—	—
I. 4	—	—	—	—	—	—	—	2	5	1	3	12	5	—	—	—	—
I. 5	—	—	—	—	—	—	—	4	5	3	11	5	—	—	—	—	—
I. 6	—	—	—	—	—	—	—	—	5	2	11	10	4	—	—	—	—
I. 7	—	—	—	—	—	3	—	1	1	9	4	2	—	—	—	—	—
I. 8	—	—	—	—	—	—	—	—	—	10	17	9	—	—	—	—	—
I. 9	—	—	—	—	—	—	—	—	—	4	14	9	5	—	—	—	—
I. 10	—	—	—	—	—	1	4	5	5	11	10	2	2	—	—	—	—
I. 12	—	1	2	2	1	—	—	—	3	—	4	8	4	1	—	—	—
I. 13	—	—	—	—	—	—	—	—	4	2	5	8	1	1	2	1	—
I. 14	—	—	—	—	—	—	—	—	7	17	3	13	6	—	—	—	—
I. 20	—	—	—	—	—	—	—	—	2	2	6	7	3	—	—	—	—
II. 1	—	—	—	—	—	—	—	—	11	9	4	3	7	—	—	—	—
II. 2	—	—	—	—	—	—	—	—	—	—	4	2	12	26	1	—	—
II. 3	—	—	—	—	—	2	2	9	7	2	9	7	14	4	—	—	—
II. 4	—	—	—	—	—	—	—	1	8	13	4	9	10	6	4	1	—
II. 6	—	—	—	—	—	—	—	3	1	7	10	6	8	1	—	—	—
II. 7	—	—	—	—	2	2	2	3	6	10	6	—	—	—	—	—	—
II. 8	—	—	—	—	—	—	—	1	4	7	7	2	3	—	—	—	—
II. 9	—	—	—	—	—	—	—	—	3	12	5	3	1	—	—	—	—
III. 1	—	1	1	2	3	6	8	17	6	4	—	—	—	—	—	—	—
III. 2	—	—	—	—	—	—	—	1	5	13	13	1	3	2	—	—	—
III. 4	—	—	—	—	—	—	—	—	10	6	4	—	—	—	—	—	—
III. 7	—	—	—	—	—	—	—	—	—	4	16	1	3	—	—	—	—
III. 8	—	—	—	—	—	—	1	6	4	4	5	4	3	1	—	—	—
III. 9	—	—	—	—	—	—	—	—	4	10	6	—	—	—	—	—	—
III. 10	—	—	—	—	—	—	—	—	2	4	4	3	6	1	—	—	—
III. 12	—	—	—	—	—	—	—	2	3	8	4	2	1	—	—	—	—
III. 14	—	—	—	—	—	—	—	—	—	5	3	1	11	—	—	—	—
III. 16	—	—	—	—	—	—	—	2	6	5	4	4	7	—	—	—	—
III. 17	—	—	—	—	—	—	—	—	4	8	6	5	1	—	—	—	—
Totals...	—	2	3	4	6	17	34	110	110	249	207	125	110	19	5	1 = 1002	—

In II. 50, the  $F_3$  parent plant of these two cultures, as shown above, the measurements of the bud cones (which are less than those of the full grown petals) ranged from 20–30 mm. in the first six flowers, the petals of the sixth (largest) flower being 42 mm. in length, and those of the

smallest probably 25–27 mm. (in the same ratio of bud-cone length to petal length). Later flowers on this plant ranged from 34–36 mm. The  $F_3$  cultures were grown in the very favourable conditions of California, in which the petals reached a maximum size. The way in which the difference in environmental conditions of the  $F_3$  in California and the  $F_4$  in England affected the size of petals generally may be understood from a comparison of the maximum size of petals in *Oe. rubricalyx*, which was 45 mm. in California and 39 mm. in my English cultures of 1920. It appears that 35 mm. was the modal length of petal for plant II. 50 grown in California. If our estimate is correct, that would be equivalent to a length of approximately 29 mm. if the plant had been grown in the conditions of my cultures in England in 1920. The modes actually observed for the two cultures 20.10 and 20.11 derived from this plant were respectively on 30 mm. and 27 mm. Hence, probably both cultures approximated to what the mode of the parent plant would have been had it been grown in the same conditions, but the offspring from the smaller flower may perhaps have significantly smaller flowers than those from the larger flower.

Figs. 9 and 10 show the curves of variability for petal-length in cultures 20.10 and 20.11. Both show negative skewness owing to the tendency to produce some small flowers, but the smallest flowers are no

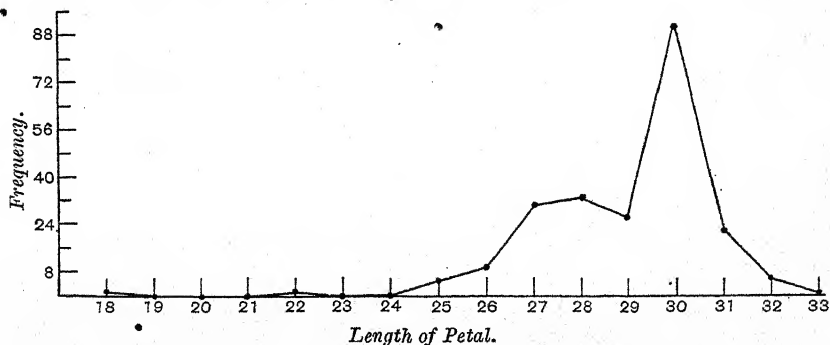


Fig. 9. Culture 10/20. Frequency Distribution of Petal Length of Whole Culture in  $F_4$ .

smaller than in *Oe. biennis*, unlike the  $F_3$  families which showed much wider variation in both directions (cf. Fig. 8). In Figs. 11–16 are given graphs for six selected individual plants in family 20.11. Comparing them with Figs. 1–7 of the  $F_2$  generation (reciprocal cross), it will be seen that although there is a distinct tendency to segregation in most plants, with negative skewness, yet the range of variation is usually decidedly less in  $F_4$  than in  $F_3$ . This is shown still more clearly by

comparing Table I with Tables II, III and IV. The range of variation is twice as great in the former table, although it only contains 35 plants while Table II contains 41. Throughout the cultures it is true that the  $F_4$  is decidedly less variable than the  $F_3$ . The same is true of the  $F_5$  compared with the  $F_4$ . This result is to be expected if the differences in size are inherited from generation to generation, even in the absence of size-units, for each culture is obtained by selfing a plant of the previous

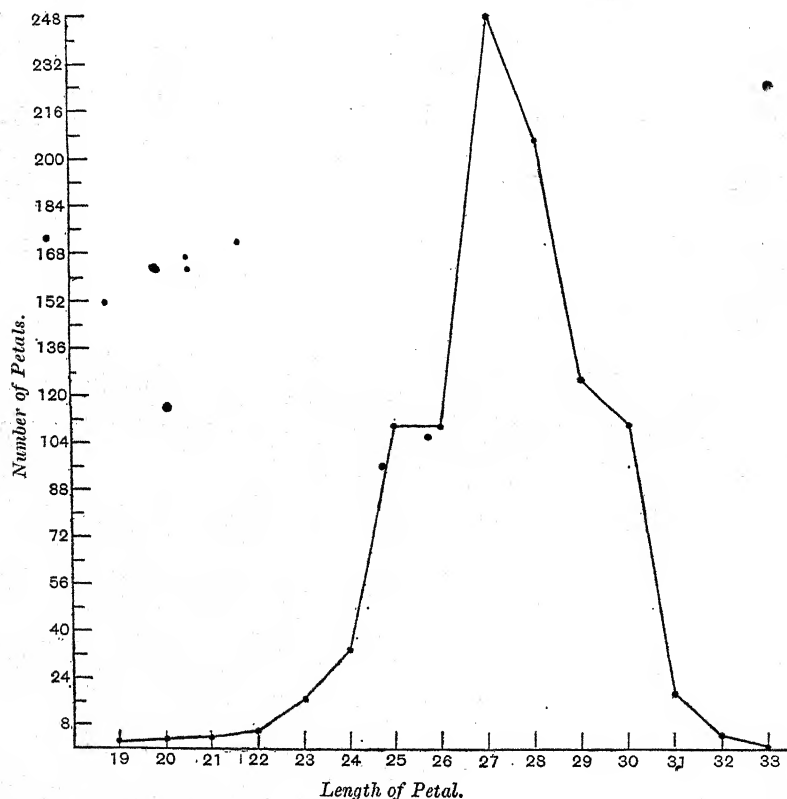
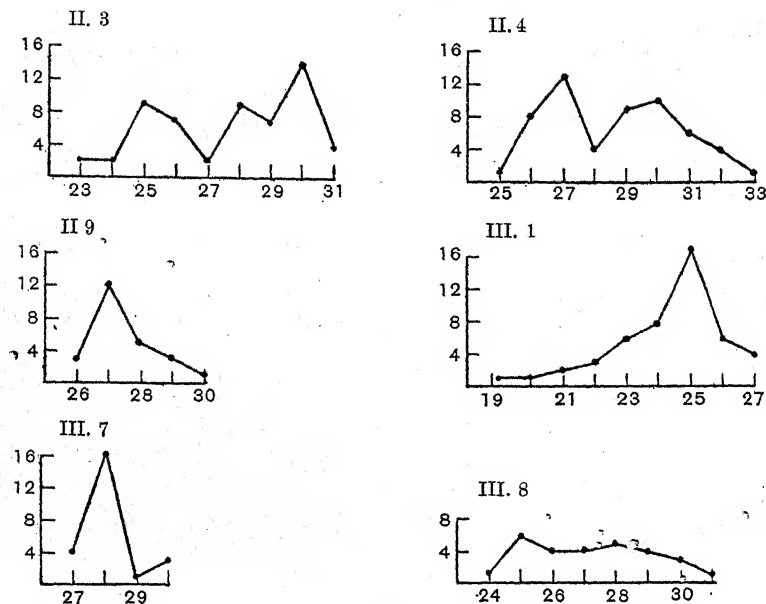


Fig. 10. Culture 11/20. Frequency Distribution of Petal Length of Whole Culture in  $F_4$ .

generation and hence practising the maximum amount of inbreeding. This is in accordance with the fact that in the absence of dominance, inbreeding may always be expected to lead to increased uniformity, on any view of heredity.

We have hitherto examined chiefly the question of segregation within the individual. Let us now examine that of genetic segregation



Figs. 11—16. Culture 11/20. Graphs of Petal Lengths of Individual Plants,  $F_4$ .TABLE III. Frequency distribution of Petal Length in  $F_4$  Family 20. 1 from Plant II. 14 in culture, 16. 72 (*Oe. rubricalyx* × *biennis*).

Culture 1/20												
Plant	18	19	20	21	22	23	24	25	26	27	28	29
I. 1	—	—	—	—	1	1	3	1	11	14	8	1
I. 2	—	—	—	—	—	3	1	12	22	7	3	—
I. 4	—	—	—	—	—	1	5	9	9	15	5	—
I. 6	—	—	—	—	—	—	—	10	7	9	10	—
I. 7	—	—	—	—	—	—	5	10	15	11	3	4
I. 8	—	—	—	—	6	12	4	8	2	—	—	—
I. 9	—	—	—	—	2	1	2	13	11	11	1	—
I. 10	—	—	—	—	—	—	2	18	7	9	—	—
I. 11	—	—	—	—	—	2	2	9	9	2	3	1
I. 13	—	—	1	—	—	3	6	9	21	27	8	1
I. 14	—	—	—	—	—	3	3	16	4	9	1	—
I. 15	—	—	—	1	5	1	4	4	4	4	1	—
I. 16	—	—	1	2	1	—	6	7	7	8	—	—
I. 17	—	—	1	3	—	2	2	5	3	—	—	—
I. 18	—	—	—	—	—	—	1	9	6	—	—	—
I. 19	—	—	2	2	1	—	3	4	7	1	—	—
II. 1	—	—	—	—	3	—	3	2	4	11	4	1
II. 3	—	—	—	—	—	1	3	4	4	1	3	—
II. 4	—	—	—	—	1	—	1	5	2	10	—	—
II. 5	—	—	—	1	1	1	3	4	2	10	2	—
II. 7	—	—	1	1	5	3	2	13	14	11	10	—
II. 8	—	—	—	—	2	5	8	14	5	9	1	—
II. 10	—	—	3	7	2	6	2	5	3	—	—	—
II. 11	—	—	1	—	3	3	4	11	6	4	—	—
II. 14	—	—	—	1	1	1	5	6	7	6	1	—
II. 15	—	—	—	—	—	—	7	12	16	1	—	—
II. 16	1	—	—	3	2	3	6	8	11	2	—	—
II. 18	—	—	—	—	3	4	2	9	2	—	—	—
II. 19	—	—	1	4	1	3	5	10	13	6	1	—
Totals	1	—	11	25	40	59	100	247	234	198	65	8=988



Table V. The difference is only 1.4 mm. and is probably not significant, especially as the family with the larger mean is derived from the mother plant with somewhat smaller mean size of petals. Moreover, the mean cannot have much significance in cases where there is a wide range of segregation in the plant. Figs. 21 and 22 show the graphs for these two

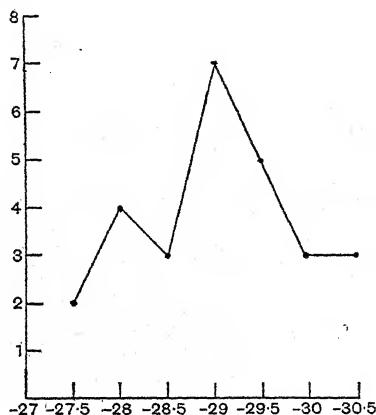


Fig. 17. Culture 10/20. Graphs of Whole Cultures from Averages of Whole Plants.

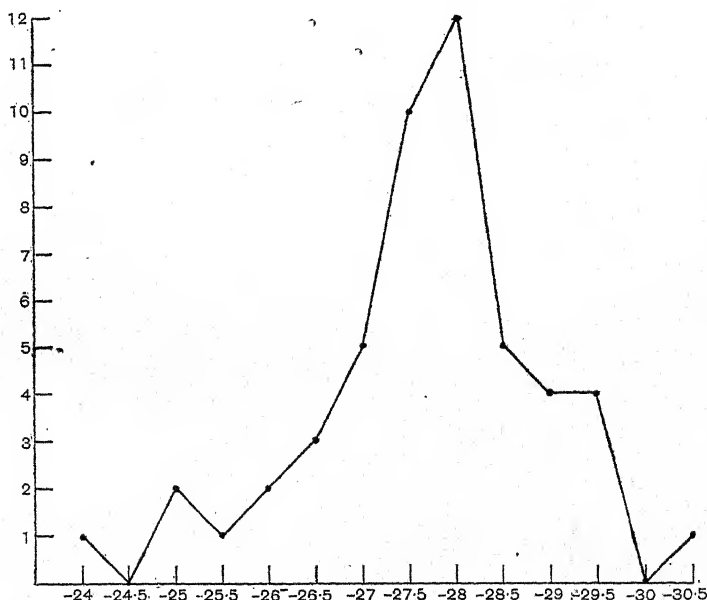


Fig. 18. Culture 11/20. Graphs of Whole Cultures from Averages of Whole Plants.

cultures based on the mean petal-length for individual plants. The tendency to segregation between individuals is clear in both curves when compared with the corresponding Figs. 19 and 20. Examination of Fig. 21 shows a tendency for modes of mean petal-length to fall at 23, 25 and 26 mm. Plant I. 8 of this culture (see Table III) for example

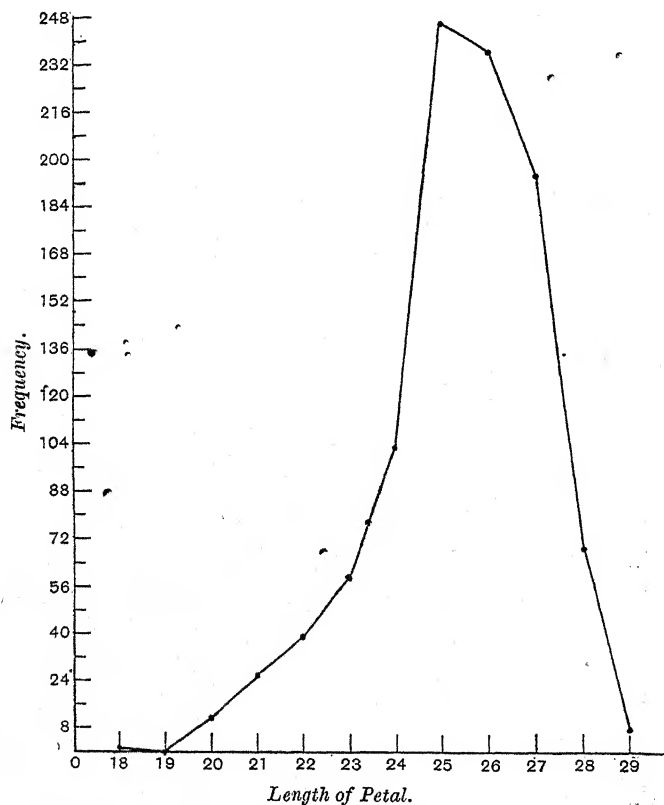


Fig. 19. Culture 1/20. Frequency Distribution of Petal Length of Whole Culture,  $F_4$ .

TABLE V.

*Means and Modes for  $F_3$  and  $F_4$  families.*

Culture	Mean	Mode	Parent plant	Mode of parent plant
16. 72	25.607	30	15. 23, III. 5	20
20. 1	25.364	25	16. 72, II. 14	28—30
20. 53	23.935	25	16. 72, II. 6	32
20. 10	28.982	30	16. 74, II. 50	35 <sup>1</sup>
20. 11	27.302	27	16. 74, II. 50	35

<sup>1</sup> Probably equivalent to 29 mm. under the conditions of culture of the  $F_4$ .

has its mode falling on 23 mm., plants I. 9 and I. 10 on 25 mm., plants II. 1, II. 5, etc., at 27 mm., II. 15 and II. 19 at 26 mm. Again in Fig. 22 we have modes of mean petal-length at 25 mm., and about 24 mm., none at 23, but one plant with flowers (four only) on 16 mm. From Table IV we

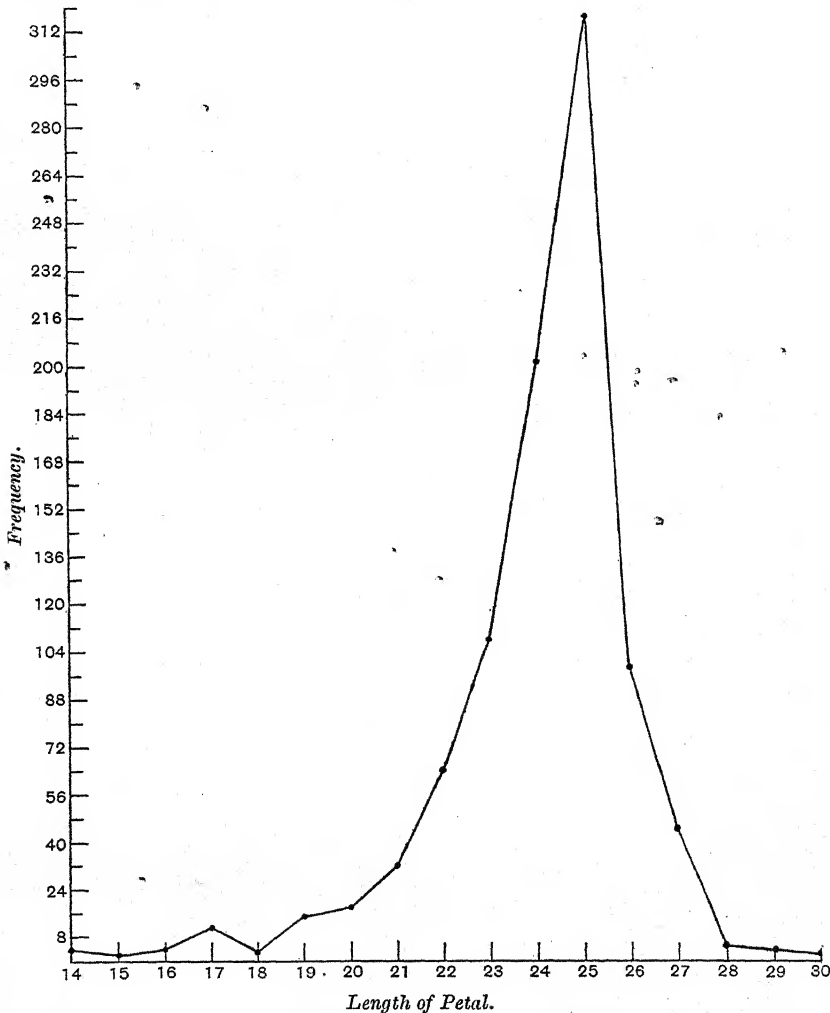


Fig. 20. Culture 53/20. Frequency Distribution of Petal Lengths of Whole Culture.

see that the mode for every plant in this culture except two falls on 23–25 mm. The parent (II. 6, Table I) grown in California had its mode at 32 mm. This, as we have seen, would be equivalent to about 26 mm.

under the conditions of the English cultures. The two exceptional plants in culture 20.53 are I. 14 with mode at 22 mm. and a peak at 25 mm., and I. 18 (only four flowers) with mode at 17 mm. Both these

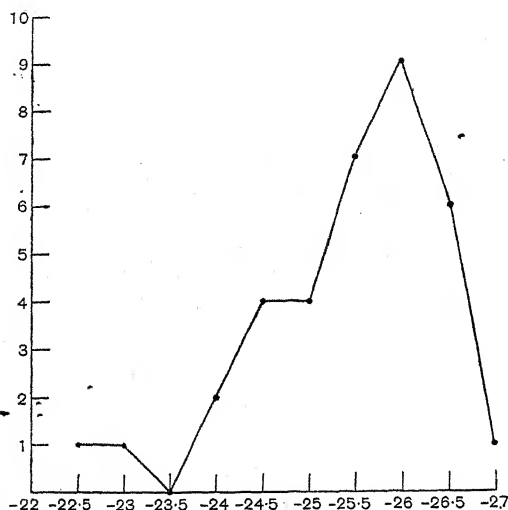


Fig. 21. Culture 1/20. Graphs of Whole Cultures from Averages of Whole Plants.

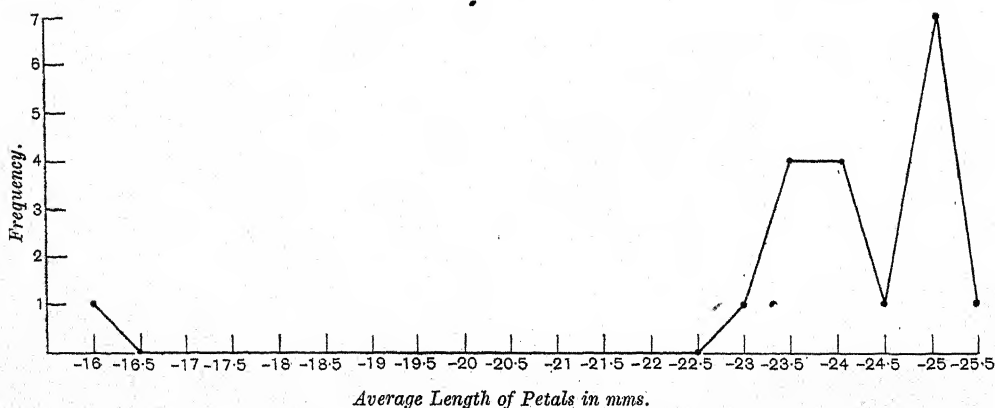


Fig. 22. Culture 58/20. Graph of Whole Culture  $F_4$  from Averages of Whole Plants.

$F_4$  families therefore tend to breed true to the conditions of the parent plant, except for the occasional segregation of plants or flowers with smaller petals. The latter occurrences become progressively less frequent in later generations.

Examination of Tables III and IV shows that while the mode is pretty uniformly on or near 25 mm., yet scarcely one of these plants shows a curve of variation corresponding with the curve of fluctuating variability. Some of them show two-peaked or even three-peaked curves, and many show negative skewness. It is clear that the behaviour is the same as in the  $F_3$  (see Figs. 1-7) only less extreme, owing apparently to the fact that they are inbred for one more generation.

Summing up the results regarding petal-length in the  $F_2$ ,  $F_3$  and  $F_4$  generations, we find segregation in various degrees, continuous and discontinuous, between (1) different plants of the same family, (2) different flowers of the same individual, and (3) different petals of the same flower. This segregation or variability however, both as regards the range of variation ("segregation") in the individual plant and the differences between plants as regards length of petals, becomes progressively less in later inbred generations, as would be expected if inherited differences were involved. This is clearly shown by comparison of the curves for  $F_3$  and  $F_4$  generations. In the  $F_4$  also the length of petals in the same flower is much more uniform. It seems that a condition of greater stability has been reached, both as regards the individual as a whole and the separate flowers. That the behaviour is not Mendelian, everyone will, I think, agree. Considered as a case of variability resulting from crossing, it clearly differs in important respects from fluctuating variability. Considered as an instance of inheritance, it differs from the Mendelian inheritance in several respects: (1) the "segregation," wherever it occurs, is not confined to the reduction divisions and appears to be in part a purely somatic phenomenon; (2) segregation may take place in any degree, continuous or discontinuous, and there is no clear evidence of fixed units. To ascribe the different sizes of petals on the same plant to "variable dominance" rather than to somatic segregation would not be satisfactory. All students of the inheritance of flower-size and of sizes in general<sup>1</sup> have concluded that there is an absence of dominance.

The differences in the range of variability and in the position of the mean and the mode in different plants of the same family (see Tables I-IV) clearly show an element of inheritance. From Table V it will be seen that while there is probably no significant difference between the  $F_4$  families from *rubricalyx*  $\times$  *biennis* there is a significant difference between them and (last two lines) the  $F_4$  families from the reciprocal cross. This may go back to an initial difference in the  $F_1$  generations.

<sup>1</sup> Except in the case of dwarfs.

The records show that the flowers were larger in *Oe. biennis*  $\times$  *rubricalyx* than in the reciprocal. A few records of petal measurements show length of petals in *biennis*  $\times$  *rubricalyx*  $F_1$  about 33 mm. and in the reciprocal "same size as *biennis*," i.e. about 20 mm. In the  $F_1$  therefore, in both crosses the flower-size was nearer that of the male parent. In the  $F_2$ , however, this difference disappears, both crosses showing much the same range, the average of the plants measured being indeed somewhat larger for *rubricalyx*  $\times$  *biennis* than for the reciprocal.

Another feature of much interest in these hybrids is the frequent occurrence of petals with slits running diagonally across them. These were referred to in my former paper (Gates 1917, p. 247) but are now much better understood. At first it seemed tempting to regard these slits as a new germinal character resulting from crossing. Van Overeem (1920) has fallen into this error, and describes a "forma *Clarkiae*" from *Oe. biennis semigigas*  $\times$  *Lamarckiana gigas*. This he figures (Fig. 2) as a case of zygomorphy with symmetrically placed pairs of slits in three of the four petals. Had he looked further he would probably have found a variety of arrangements of these slits, which have nothing to do with zygomorphy in the ordinary sense and are seldom arranged in the symmetrical way he figures. Van Overeem explains their occurrence as the appearance of a latent character in connection with the anomalous chromosome numbers in his hybrid. That this is not the explanation is shown by the fact that such slits occur, probably much more frequently, in the hybrids described in this paper, where the chromosome numbers are 14 in both parent species and probably also throughout the hybrids, except for the very occasional occurrence of mutants with other numbers.

The significance of these peculiar slits was discovered in 1920. They occurred very commonly in all the  $F_1$  cultures, although they were not usually so long as in the  $F_2$ . They were nearly always mirror images of each other. In Fig. 23 is given a set of natural-size drawings of the petals of four flowers from the  $F_1$  populations. Records were kept of hundreds of flowers in which not only the length of petals but also the position and length of these slits were recorded. The slits varied much in length but the two forming a pair in adjacent petals were nearly always of the same length. Sometimes, however, where a slit developed in one petal, the mirror-image position at the angle of the next petal was crumpled by pressure but without a slit. This fact together with the interlocking of the slits in adjacent petals in the buds, led to the discovery of the real nature of this "new character." It is not due to a germinal change, although the slits have been commonly observed



in  $F_3$  and  $F_4$ , nor is it the patency of a latent character in any useful sense. The condition apparently arises from the difficulties of adjustment in crosses between forms with large and small petals. As a result the young petals are more or less crumpled in the bud<sup>1</sup>, and owing to mutual interference or pressure, these slits develop. They occur in flowers of all sizes, and apparently quite as often when all the petals of a flower are the same size as when they differ in length. The failure of adjustment is then a deep-seated one in the hybrid race. Quite frequently two pairs

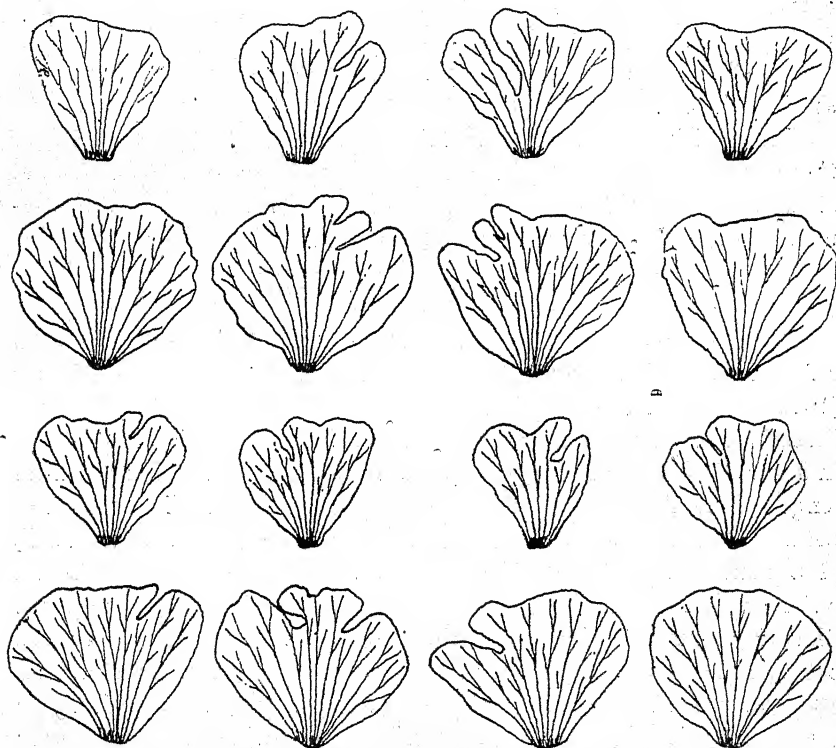


Fig. 23. Petals of four flowers (horizontal rows) showing mirror-image slits.

of slits (parallel) develop in adjacent petals, but oftener only one. In length they vary from 1 mm. or less to 18 mm., extending right across the petal. For a further understanding of this anomaly an investigation of the organogeny of the flower would be necessary. Failure of adjustment between primordia owing to conflicting inherited tendencies appears to be as far as it can be traced now. Like the size-variations,

<sup>1</sup> In how far this may be due to smaller sepals enclosing larger petals is uncertain.

these slits were more frequent and of greater length in  $F_3$  than in later generations, but they still occur as quite small and infrequent slits in this season's  $F_6$  cultures. We have recently found that they are not entirely absent from some of the larger-flowered pure species, but when they occur at all they are small, inconspicuous and infrequent.

Davis (1913), in his studies of hybrids between *Oe. biennis* and *Oe. grandiflora*, noticed that there is a "decided segregation of flower size" in some of the  $F_2$  hybrids. He observed the "very common cutting of the petals at the edge into narrow segments as in lacinate varieties of flowers," and speaks of a general tendency to progressive increase in size, but he apparently made no further attempt to analyze the inheritance.

I am indebted to my Research Assistant, Miss E. M. Rees, B.Sc., for these drawings of petals and also for preparing all the graphs and several of the tables in this paper, as well as making some of the petal measurements in the  $F_4$  generation.

#### *Discussion.*

Some of the studies of size inheritance in plants have already been referred to. We may now consider an interesting paper of Tine Tammes (1911) in which the inheritance of a number of size characters was investigated. Here we may refer particularly to her study of petal-size in flax varieties. In reciprocal crosses between Egyptian flax (medium length of petal 16.2 mm.) and *Linum angustifolium* (medium length of petal 8.08 mm.), the  $F_1$  of 9 plants was relatively uniform, while the  $F_2$  (203 plants) showed the whole range of variation to the parent types, but there is no mention of different sizes of flowers on the same plant. Similar results are given for breadth of petal. In reciprocal crosses between Egyptian and ordinary flax, however, in which the  $F_1$  numbered 33 plants and the  $F_2$  families 120, the variation in  $F_1$  was practically as great as in  $F_2$ . Some of the  $F_3$  families from this cross were nearly uniform, others showed nearly the whole range of variation present in  $F_2$ . In certain other crosses the variation of the  $F_1$  was nearly as great as in  $F_2$ . The writer concludes that very probably the difference between the flower size of *L. angustifolium* and "ordinary flax" is represented by three or four units. In the light of the present results it seems doubtful whether definite size-units are involved at all in crosses of this type of character.

East (1913) has made a similar, but more detailed, study of flower-size inheritance in *Nicotiana Forgetiana*  $\times$  *N. alata grandiflora*. In the

former species the mean length of corolla was 25.6 mm., and in the latter 78.8 mm. The  $F_1$  showed a mean value of 44.3 mm. with very little variability, and the  $F_2$  a greatly increased variability. The spread of the corolla was said to behave in the same way. Fig. 24 shows the curve of variability for length of corolla in  $F_2$ , from East's data (Table IV), for comparison with such curves as Fig. 8 of the present paper. The

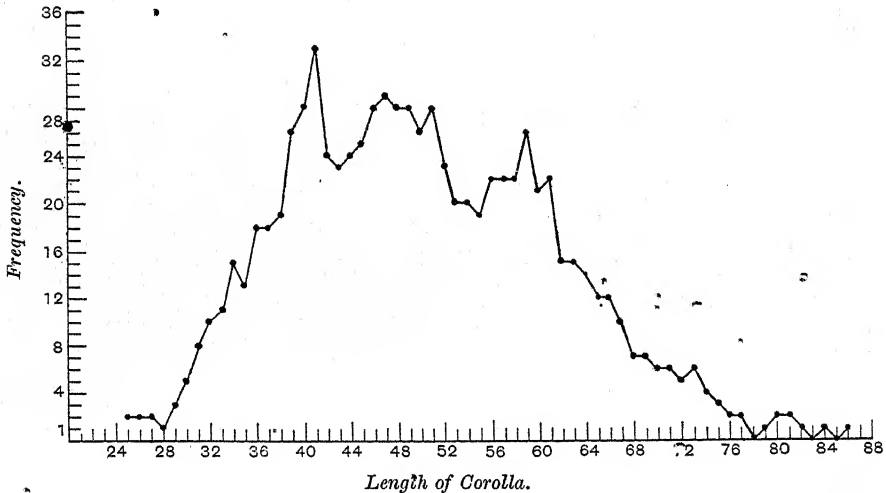


Fig. 24. Frequency Distribution of Corolla Length in *Nicotiana Forgetiana*  $\times$  *N. alata grandiflora*,  $F_2$ . After East, from his Table IV.

curves appear in some respects comparable, although the skewness is in opposite directions. In East's data, however, the measurements for each family are treated as a whole. No records are given of the range of variation on individual plants or the differences between sibs of the same culture, nor is it stated how many flowers were measured from each plant. If such records had been kept they might have shown results similar to those described in the present paper, although it is probable that the range of variation on the individual was not conspicuous, as in *Oenothera*, or it would certainly have been noticed even by a casual inspection.

In a later study of crosses between *Nicotiana Langsdorffii* and *N. alata*, East (1916) finds the  $F_2$  generation (corolla length) again nearly three times as variable as the  $F_1$ , with certain  $F_2$  individuals reproducing a *Langsdorffii* population in  $F_3$ . "Individuals from the same point on the  $F_2$  curve showed different variabilities in  $F_3$ ." Yet notwithstanding this statement in the conclusions, no separate measure-

ments of the flowers of individual plants are given in the paper. Whether the results are really the same as in *Oenothera*, *i.e.*, with often a wide range of flower-size on the same plant, can only be determined when such records for individual plants are kept. East's conclusion that "Mendelian inheritance seems to be the *only* (his italics) logical interpretation" of these phenomena, must clearly be held in doubt. Indeed, the most reasonable interpretation appears to be that the phenomena of inheritance of petal-size in *Oenothera* are not unique, and that flower-size in other plants, and perhaps sizes of repeated parts or organs in general, when similarly analysed will be found to show similar hereditary behaviour. At any rate, it is clear that while an increased variability of the  $F_2$  over the  $F_1$  indicates some form of segregation in  $F_2$ , it does not necessarily show Mendelian segregation of definitely fixed units in the  $F_1$  germ cells. These studies of petal-length in *Oenothera* hybrids on the contrary prove that somatic segregation on a large scale is occurring in the  $F_2$  and later generations. The results, however, indicate also a tendency to germinal segregation between different plants in  $F_2$  and following generations.

Goodspeed (1912, 1913) has made extensive studies of flower-size in tobacco. He crossed three varieties of *Nicotiana acuminata* which differed only in flower-size, having mean corolla diameters of 27, 20, and 13 mm. respectively, with fluctuations never exceeding 2 mm. greater or smaller than the mean diameter in each case. In crosses between these types the variation in corolla diameter was greater in  $F_2$  than in  $F_1$ . But certain of the  $F_2$  plants which bear the largest flowers exhibit as small ranges of variation as the original parents. The  $F_1$  plants, however, showed a range of variation 2 or 3 times as great as that of the parents, while the maximum range in  $F_2$  was 5 or 6 times that of the parents. Goodspeed concludes that the results "preclude the possibility of a simple Mendelian interpretation." In a later paper, in which more extensive data are available, Goodspeed and Clausen (1915) record measurements of flower-size in various hybrids, including (*N. Tabacum* "Maryland"  $\times$  *Tabacum* "Cavala")  $\times$  *N. sylvestris*. The frequency distributions for corolla spread are given for individual plants. They show in most plants a long range of variation in corolla spread, but with usually a single mode. In a few plants, however, the range is narrow. The authors controvert East's statement that flower-size is independent of environmental conditions, but admit that flower-size is less markedly modified by environment than height or leaf-size and other characters. They find that length of corolla is more stable than spread, which is rather readily

subject to modifications "under stress of internal and external conditions of development."

So far as the data are available, they indicate a condition of variation in the individual comparable with that described in *Oenothera*, the only apparent differences being that the variation is less conspicuous and it begins in the flowers of the  $F_1$  hybrids. The  $F_1$  in *Oenothera* hybrids is on the contrary relatively uniform in flower-size, and measurements made in 1920 on the flowers of ten plants from the  $F_1$  of *Oe. Novae-Scotiae*  $\times$  *Oe. rubricalyx* showed remarkable uniformity not only in flower-size but in every other character. The parent species grown under the same conditions had petal lengths respectively 12-13 mm. and about 40 mm. In the  $F_1$  hybrids the flowers on the main stem had petals uniformly 18-20 or 22 mm. (mean 20) in length, while the flowers on the lateral shoots, although blooming at the same time, were uniformly smaller, measuring 15-18 mm. (mean 16). This confirms the statement for other *Oenothera* hybrids that the  $F_1$  is uniform as regards size of flowers.

Viewing the whole of the results as regards flower-size in these *Oenothera* hybrids, we find a nearly intermediate condition, with uniformity, in  $F_1$ , followed by wide segregation with an even wider range of variation in  $F_2$  than in the combined parent forms. The remarkable variation in different flowers of the same plant, and even in different petals of the same flower, is the most striking feature of the  $F_2$ . These phenomena cannot be explained on a Mendelian basis, and lead to the probability that flower-size in other plants, and perhaps sizes in general, where repeated in the same organism, are not Mendelian in their inheritance. In any case the numerous instances of size-inheritance which have been interpreted in general Mendelian terms cannot be accepted as proven to be Mendelian until much more carefully analysed data are presented in their support.

In  $F_3$  and  $F_4$  and later generations the *Oenothera* hybrids showed progressive diminution in the range of variability, until in some plants the curve of variation is scarcely distinguishable from an ordinary curve of fluctuation. It is probable that many similar cases in plants, which have been interpreted as mere fluctuating variability, are in reality the result of earlier crossing between forms which exhibit germinal differences as regards flower-size. The phenomena as a whole must be looked upon as a distinct type of variability. The occurrence, probably through mutation, of varieties differing only in size of flower is known in many species of plants. Still more common is the occurrence of species in

the same genus which differ in flower-size yet which are capable of intercrossing. It is probable that any population of plants in which there appears to be wide fluctuation in flower-size is really the result of complex crosses among the descendants of varieties showing originally a marked inherited difference in flower-size.

It has already been shown (Gates 1917, p. 248) that the wild *Oenothera Hookeri* (*Oe. Franciscana*) which occurs at Lake Merced, California, contains occasional small-flowered plants in addition to those having large flowers, and that some of the latter when observed in cultivation show a wide range in length of petals on the same plant, just as in the *Oe. biennis-rubricalyx* hybrids. There is no reason to doubt that the explanation is the same, i.e., it is due to an earlier cross in the population between large- and small-flowered types. There was this interesting difference, however, in the *Oe. Hookeri* hybrids. The differences in flower-size on any plant were almost entirely between different side shoots of a plant, and rarely if ever between different flowers of the central stem, while in the *Oe. biennis-rubricalyx* hybrids the measurements were chiefly confined to the flowers of the central stem where these size-differences occurred.

So far as I am aware, no other case of variability precisely similar to this has been investigated in plants or animals. The cruciate type of petal in *Oenothera* appears, however, to be essentially similar in its hereditary behaviour, although this may be regarded as a qualitative character, but its variability in crosses begins in the  $F_1$ . There can be little doubt that the form which De Vries (1903) described as *Oenothera cruciata varia* from gardens arose from a cross between cruciate and broad-petalled forms. He found that in culture some plants produced only cruciate, some broad-petalled flowers, while others produced a varying array of intermediates. In certain flowers both broad and narrow petals occurred in the same flower, or even the two halves of the same petal might be respectively broad and narrow, thus paralleling in kind the size-variation described in this paper. De Vries has referred to the same behaviour in crosses of *Oe. Lamarckiana* and *Oe. cruciata* (1913, p. 156), and in *Oe. muricata*  $\times$  *cruciata* (*l.c.*, p. 77). In the latter case, which is more fully described, the  $F_1$  flowers showed all intergrades between broad and narrow petals as well as plants which were predominantly broad- or narrow-petalled, thus differing from the  $F_1$  between large and small petals, which in *Oenothera* appears always to be uniform. In later generations similar conditions of intermediacy were obtained.

The various cruciate types of *Oenothera* have no doubt originated

as parallel mutations in different species (see Gates 1915, p. 21 and 1921, p. 48). A cruciate variety of *Epilobium hirsutum* behaves as a simple Mendelian recessive when crossed with the species from which it mutated, and it is quite possible that each cruciate variety of *Oenothera* would do the same when crossed with the form from which it arose. In species crosses, however, we get irregular behaviour in  $F_1$  or  $F_2$  with both these characters. In the main essentials, length of petals and breadth of petals usually then behave in the same manner in crosses. The *Epilobium* case indicates that a simple variety arising through a mutation will behave as a Mendelian difference, while the specific size-differences involved in these crosses behave in the peculiar irregular manner here described. In the *Oe. Hookeri* (*Franciscana*) hybrid above mentioned, however, there was no indication of a species cross in the population. In any case this peculiar behaviour is a phenomenon of incompatibility, and in the majority of cases at least it occurs in species-crosses, *i.e.*, crosses in which something much more complicated than single mutational differences<sup>1</sup> is involved.

This brings us to the question of incompatibility between characters in crossing. Davenport (1917) maintains that some of the factors controlling stature in man are general growth factors affecting the whole body, while others are local size-factors determining the length of particular bones or segments of the body. He also believes that in racial crosses through recombinations of such characters, disharmonies may arise, such as large teeth in small jaws or a small heart with a large blood system. Castle (1922), on the contrary, maintains from his studies of size-inheritance in rabbits, that all size-factors in animals are general growth factors, though he expressly excludes plants from this conclusion. Without entering into criticisms of the relative merits of the data on which these two views rest, we may be permitted to make two remarks: (1) we see no *a priori* reason why there should not be factors controlling chiefly the size of particular organs, just as there are many qualitative factors affecting chiefly the colour or shape of particular organs; giant varieties of many fruits are known, in which only the size of the fruit appears to be affected; (2) there may be an important difference as regards inheritance, between the sizes of repeated parts such as the flowers of plants, and of single parts such as general bodily size or weight or the dimensions of their constituent elements.

As regards disharmonies, it is certain that they occur in these hybrid

<sup>1</sup> This statement applies of course only to those mutations in which no visible change in the chromosomes is involved.

Oenothera flowers. Most frequently when a conspicuously small flower occurs among larger ones on a plant its petals are approximately equal in length. The change, whatever it is, which gives rise to the smaller flower, must then be thought of as occurring in the production of that flower primordium. The flower then develops as a unit and there is no apparent disharmony, the sepals being correspondingly smaller so as to enwrap the petals properly, and in extreme cases the hypanthium and ovary being reduced in length and thickness as well. Where long and short petals occur in the same flower there must have been "segregation" of some sort between the different petal primordia, or at any rate between the four sectors of the flower. Extreme cases of this kind have been few, and further observations are required to determine whether in these cases the sepals always correspond in length to the petals opposite them, or whether they may independently behave as a unit when the petals are of unequal length.

Reference must here be made to another paper which bears on the general theory of cumulative size factors. Sumner and Huestis (1921) have made measurements of the relative lengths of corresponding right and left bones, in connection with their breeding experiments with the Californian Deer-mouse, *Peromyscus maniculatus*. For instance, they measured the right and left mandible and humerus, determined the sinistro-dextral ratio in each case and showed statistically that there is no tendency to the inheritance of this ratio; for example, if a mouse has a larger left humerus than right its offspring do not inherit this condition. Yet these authors have shown that in crosses between different sub-species of this mouse, the  $F_2$  shows a significantly greater range of variation than the  $F_1$  with regard to these sinistro-dextral ratios. From this it follows that a mere increase in range of variation of the  $F_2$  over the  $F_1$  cannot be regarded as sufficient evidence of the presence of plural size factors.

### Conclusions.

In this and a previous paper a study is made of the inheritance of size of petal in four generations of reciprocal crosses between *Oenothera rubricalyx* and *Oe. biennis*. The results as regards petal-size differ in several respects from anything previously described. A uniform and more or less intermediate  $F_1$  is followed by wide segregation in  $F_2$  not only between different individuals but also as regards the length of petals on the same plant, and even in the same flower. The smallest flowers obtained had petals less than half as long as the smaller parent



(*biennis*): the largest flowers equalled, but did not exceed, those of *rubricalyx*. This segregation may be continuous or discontinuous, and appears to be quite haphazard, often resulting in a two or three peaked curve for a single plant, larger and smaller petals occurring intermingled, sometimes even in the same flowers. In the  $F_2$  and  $F_4$  similar phenomena of segregation or variability occur, but they are much less marked in  $F_4$  than in  $F_2$ .

The various graphs of variability show that there is segregation, both germinal and somatic, but the data are insufficient to determine the precise relationship between these two. As regards the segregation between individuals, the evidence does not justify the assumption of several fixed Mendelian size-factors, and it is evident that the whole problem of the inheritance of cumulative size-factors needs re-examination. It appears that in later generations the modes of petal-length for individual plants may occur at any point, and there is no evidence that they tend to occur more frequently at one point in the series than at another, nor is there any evidence that the somatic segregation tends to occur at certain points in the series and not at others.

Viewed as variability, this behaviour differs from fluctuation in two respects: (1) a variety of curves are obtained for different individuals, which seldom if ever conform to the curve of fluctuating variability, (2) the condition has apparently arisen through failure of adjustment between different size-tendencies inherited from the original cross. The disharmony expresses itself in petals of different sizes sometimes occurring in the same flower or more frequently in flowers of different size on the same plant. The frequent occurrence of slits in the petals appears to be due to mutual pressure in the bud producing these mirror-image phenomena. It is not a new germinal character but is rather another result of disharmony.

This type of variability stands between ordinary Mendelian inheritance on the one hand and fluctuations on the other, having certain features of both but differing from either in its irregularity.

Large- and small-flowered forms occur in many related species and varieties of plants. If crossing and back-crossing occurred between them, a population would result in which there was a considerable range of variation, which would have the superficial appearance of fluctuation but would in reality be this type of inheritance and variation following crossing. Probably many cases of size variation in plants took their origin in this way. This is illustrated by a wild population of *Oenothera Hookeri*.

Since Mendelian behaviour is now generally admitted to be based upon the segregation of chromosome pairs in the reduction divisions, it appears probable that the phenomena of variability and segregation here described are based partly upon cytoplasmic differences between the parental forms. This would account for their irregular character, since there is no definite cell mechanism comparable with the mitotic figure, for distributing cytoplasmic structures or substances.

It appears at present inadvisable to consider Mendelian behaviour as demonstrated in any case in which the  $F_2$  cannot be individually classified into distinct categories on the basis of their visible differences, unless clear evidence of the segregation of *fixed* units can be obtained from the  $F_3$  or later generations.

In conclusion I wish to record my thanks to the Royal Society and the British Association for Grants in connection with these researches, and to the Royal Botanic Society, Regent's Park, for the facilities provided.

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# ON THE NATURE OF THE CENTROSOMAL FORCE.

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(With Four Text-figures.)

IN 1908 A. B. Lamb, in a paper entitled "A New Explanation of the Mechanics of Mitosis," put forward a hypothesis, in which he suggested that the force, of which the mitotic figure is an expression, might be "those mutual repulsions and attractions, exerted by bodies pulsating or oscillating in a fluid medium."

It was shown by Bjerknæs ('00), father and son, that in any fluid medium, if any two bodies are pulsating synchronously and in opposite phase they repel one another, or if they are oscillating synchronously and in the same phase they similarly repel one another. In both cases there is a field of force set up between the pulsating or oscillating bodies which is identical in form with that between unlike magnetic poles which, of course, attract one another. Lamb accordingly suggested that during mitosis the centrosomes are bodies pulsating synchronously and in opposite phase or oscillating synchronously and in the same phase. He pointed out that this would result in a field of force which would agree with that observed in a mitotic figure, while at the same time it would explain the moving apart of the two centrosomes. Cases of multipolar spindles would be impossible if the centrosomes were pulsating bodies but could easily be explained if it was assumed that they were bodies oscillating synchronously and in the same phase in paths radial to a common centre.

The movements of the chromosomes would also be adequately explained on the same hypothesis, for Bjerknæs showed, so Lamb ('08) states, "that bodies suspended within the field of force of oscillating or pulsating bodies are attracted or repelled depending upon whether they are lighter or heavier than the surrounding medium." Thus the chromosomes, if heavier than the surrounding medium, would be repelled from the centrosomes and so come to occupy a position midway between them in the equatorial plate. They would remain here grouped together

by mutual attractions resulting from induced oscillations. A change in density in the chromosomes after splitting would be sufficient to cause them to diverge back towards the centrosomes.

Before proceeding further, it must be pointed out that Lamb misquoted Bjerknæs, in his statement of the attraction or repulsion of bodies suspended in the fluid medium by the oscillating body, according to the density of those suspended bodies. Bjerknæs ('00), referring to the force causing this attraction or repulsion, states: "Die Kraft ist abstoßend, wenn die neutrale Kugel leichter, dagegen anziehend wenn sie schwerer als die Flüssigkeit ist," which is the opposite of Lamb's statement. This mistake, which has been copied by Prenant ('10) and D'Arcy Thompson ('17) does not however materially affect Lamb's hypothesis. The main fact remains, that, whether the chromosomes will be repelled or attracted by the centrosomes will depend on the relation between their density and that of the ambient fluid.

Bjerknæs' calculations were based on the theoretical consideration of the oscillations of perfect spheres in perfect fluids, but in the experimental volume of his work he showed that actually one can obtain these fields of force in fluids by the oscillations of solid spherical bodies. Hence Lamb was perfectly justified in assuming that Bjerknæs' theory would apply generally to oscillations in the fluid contents of the cell. He points out that some of the formulae deduced by Bjerknæs cannot be strictly applied to oscillations in a field which is both viscous and heterogeneous, and no attempt is here made to apply any of these formulae strictly. Merely the main results of Bjerknæs' theory, as applied by Lamb to the Mechanism of Mitosis will be discussed.

Lamb's hypothesis has met with scant criticism, D'Arcy Thompson ('17) refers to it as a "novel and elegant hypothesis," Prenant ('10) merely states that the assumption of a periodic change in the density of the chromosomes is not supported by any fact, Spek ('18) dismisses the hypothesis with the statement that an oscillating body can scarcely play a rôle in cell division. Hartog ('14) mentions Lamb's paper in his list of references, while Meek ('13) in a review of the theories of mitosis does not mention it at all. It is extraordinary that this concise paper has received so little attention. The only real objections, those of Prenant ('10) and Hartog ('13), it will be more convenient to deal with later.

Lamb puts forward his suggestion as "an *ad hoc* constructed hypothesis and intrinsically therefore only of hypothetical value." The object of the present paper is to show that, assuming this hypothesis to be correct, there must follow several consequences which it is maintained

possibly explain certain phenomena of cell division and fertilisation which otherwise remain obscure.

The nature of the force which is centred at the centrosome cannot be elucidated from the study of any single cell or type of cells. It is obvious that in some cells this activity is less obscured than in others. The method of this paper is to apply Lamb's hypothesis to the case of an hypothetical, isolated, ideal cell and then to apply the deductions made from this consideration to actual described cases.

In any emulsion, in the sol state, the particles constituting the disperse phase normally remain uniformly distributed throughout the continuous phase of the emulsion. If now there is, in that emulsion, an oscillating body, then particles will be attracted to or repelled from that centre of oscillation according as their density is greater or less than that of the continuous phase.

Let us suppose that the density of the disperse particles is smaller than that of the continuous phase. They will then be repelled from the oscillating body. The force with which any individual particle is repelled will depend upon its distance from that oscillating body. Bjerknes states that the force will vary inversely as the seventh power of the distance. Thus a particle situated at a short distance from the oscillating body will be repelled with a force much greater than that acting on a particle situated a little further off. However, as the particles move outward they are moving continually into a greater volume but this volume only increases as the second power of the distance from the oscillating centre while, as stated above, the repellent force varies inversely as the seventh power of this distance. This means that those particles near to the oscillating body will acquire comparatively, a much greater acceleration than those situated further out, and therefore will catch up to, and crowd upon the latter. Hence, as the particles move outwards not only will there be formed round the oscillating body a sphere of continuous phase from which the disperse particles have been forced outwards, but immediately outside this sphere there will be a zone in which there is an accumulation of the disperse phase in a concentration much greater than that of the undisturbed emulsion. Outside this zone the concentration of the disperse phase will diminish with the distance from the centre, gradually approaching the normal concentration.

If that emulsion is in the form of a drop and the oscillating body is at its centre then there will be another zone of increased concentration of the disperse phase at the surface of the drop, for the disperse particles on reaching the surface will be retained there by the surface film. In

Fig. 1 the line  $AB$  represents the normal ratio of continuous phase and disperse phase in the undisturbed emulsion while the curves  $r_1t_1$ ,  $r_2t_2$ , and  $r_3t_3$  represent the same ratio at different distances from the centre of oscillation at intervals  $t_1$ ,  $t_2$  and  $t_3$  after the oscillating body has commenced to oscillate.

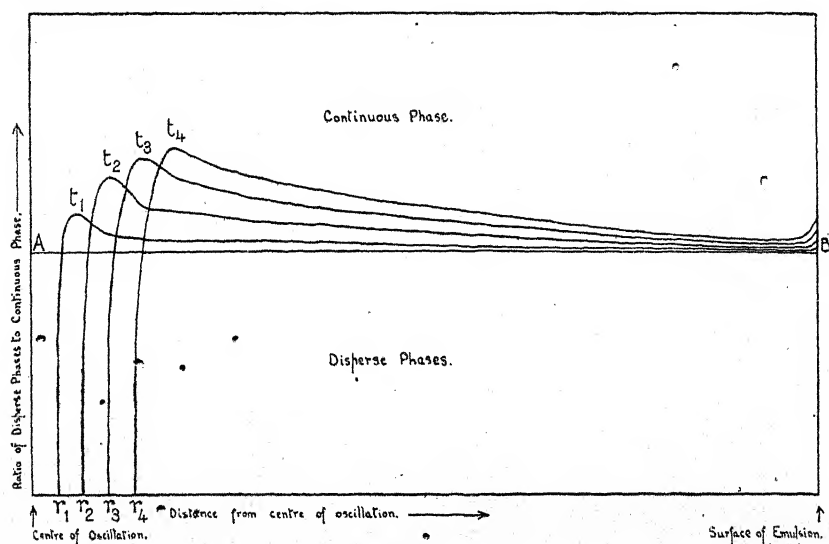


Fig. 1.

The viscosity of an emulsion depends, among other things, upon the number of disperse particles that exist in a unit volume of the emulsion. Hence in the condensed zones surrounding the oscillating body there would be increased viscosity, while in the sphere immediately surrounding the centre there would be comparatively very low viscosity, namely that of the continuous medium.

The repulsion outwards of the lighter phase will necessitate a flow of the continuous phase towards the centre of oscillation to take the place of the outgoing particles. Thus one would expect to find a streaming movement towards the centre. Also this flow would suffer the least resistance if it were concentrated in distinct radial channels.

This consideration does not apply solely in the case of a pure emulsion but would apply equally well if there were scattered through that emulsion any other particles of larger size than the disperse particles of the emulsion.

These theoretical effects that should result from the oscillation of a small body in an emulsion agree closely with Chambers' ('17) observa-



tions on the formation of the aster in the egg of *Echinarachnius*. He describes the sphere—the central portion of the aster—as consisting of liquid free of granules, and states that it increases in size until the aster attains its full development. The increase in size is due to an accumulation of liquid from all parts of the egg—this liquid streaming along channels towards the centre of the sphere, along the so-called astral rays. Chambers further states that the “cytoplasm between the rays is in the gel state....The gel state is most pronounced near the sphere, and peripherally passes gradually into the sol state of the cytoplasm lying beyond the confines of the aster.” Chambers’ use of the word “gel” is apt to be misleading, and this will be discussed later. It is not clear upon what criterion he based his definition of a gel, but from the later work of Seifriz ('20) it appears that the cytoplasm making-up the wedges between the centrally directed streams can be stated merely to be in an exceedingly viscous state. (See p. 61.)

In order that this similarity between the observation of Chambers and the theoretical results of Lamb’s hypothesis may hold good, it is not necessary to consider protoplasm simply as an emulsion in which the density of the disperse phase is less than that of the continuous phase. All that it is necessary to assume is that, in such an egg as that studied by Chambers, while the aster exists as such, all the disperse particles surrounding the centrosome have, on the whole, a density less than that of the continuous medium. Bayliss ('19) states that protoplasm is “an extraordinary complex heterogeneous system of numerous phases and components.” There will be particles lighter than the continuous phase and there will also be particles more dense. If, in the mass of protoplasm from which the aster is formed the lighter particles predominate, the efflux will be more considerable than the influx and hence the denser particles will be mostly carried outwards with the lighter particles. This will lead to the highly viscous zone being formed round the centrosphere, and further, this zone will protect the centrosome from the denser particles that may gradually find their way towards the centre of the aster from the most outlying cytoplasm. Thus at the full development of the aster, one would expect to find, to some extent, an accumulation of those particles more dense than the continuous phase on the outside of the highly viscous zone of the aster but not in the centrosphere itself. The aster of a sea-urchin egg is the only one yet described by the aid of microdissection. Other types of asters, which in fixed preparations show a grouping of particles immediately round the centrosomes as in certain spermatoteleotic mitoses could be feasibly explained

on the assumption that these were "heavier" particles that had "got through" to the centrosome before the highly viscous zone was fully formed.

It will be taken for granted that the formation of the amphiaster is a result of the division of the single centrosome in the single aster. This division results in the production of two oscillating bodies which repel each other and which are situated in the centrosphere. Now the latter consists of continuous phase and will therefore be comparatively fluid. Hence the two centrosomes will at once repel each other to the periphery of the centrosphere, that is, into the highly viscous layer. But this viscous zone is repelled by the centrosome. Further, in the aster, the astral rays are the steady stream lines towards the centrosome. Obviously then, the existence of two centrosomes at diametrically opposite parts of the centrosphere will cause a complete reorganisation of the materials forming the single aster. These will reconstitute themselves round the two centrosomes, when these have attained their maximum distance apart (see p. 58), the stream lines converging now towards the two separate centres. Thus the formation of an amphiaster from a single aster will not take place by the separation, intact, of two halves, each intact, of the single aster but will consist of a complete breakdown of the aster and its reconstitution about two centres. Again this is what Chambers states to be the case in the formation of the amphiaster in *Echinarachnius*.

It is not possible to predict much as to the arrangement of the phases in the spindle region, that is, in the region between the two centrosomes. At a point equidistant between the two centrosomes a lighter particle will be acted upon by two equal and opposite attractions and the resultant force acting upon it will be zero in the direction of either centrosome. The force gradient between the two centrosomes therefore will be more steep than that between the centrosomes and the outlying parts of the cytoplasm, and it is the force gradient that causes the production of the viscous zone. But, on the other hand, the spindle does not arise in an undisturbed mass of cytoplasm, it arises in the region occupied by the centrosphere of the single aster—and this is a region consisting of continuous phase containing very little of the lighter disperse phase. Hence if the two centrosomes are near enough, compared with the size of a single aster, one can predict that the spindle region, at the time of its formation, will be fluid as it was found to be by Chambers ('17) and Seifriz ('20). If the centrosomes are comparatively far apart, the influence of either on the

cytoplasm surrounding the other will be small and so there will be formed round each an aster, and these will be practically independent of each other. Such an arrangement is often figured as for example in the pro-phase of the first cleavage of the egg in *Ascaris megalocephala*, Boveri (1887).

In the unequal cleavage of *Crepidula* Conklin ('12) states that "The centrosomes and spheres are the cell constituents which first become unequal....As soon as the spindle becomes excentric the centrosome and sphere which lies the farthest from the centre of the cell becomes smaller than the one at the opposite pole." This is what might be expected. In the smaller potential blastomere the surface will be brought nearer to its centrosome and so the crowding of the lighter material in the zone around the centrosphere will be assisted and so will occur nearer to the centrosome. Thus the centrosphere will become smaller. Conversely the sphere in the other half of the egg should enlarge.

Up to now the activity of the centrosome has been considered more especially as affecting only the cytoplasm. It should however be possible to demonstrate its effect on the cytoplasmic inclusions of the cell such as the mitochondria, yolk, etc. These bodies should arrange themselves around the centrosome as focus. In many figures of fertilisation and mitosis this concentric arrangement is obvious. A striking example showing such an arrangement is figured by Vejdovsky and Mrazek ('03) in the pro-phase of the early blastomeres of *Rhynchelmis*. Here at least five distinct zones can be seen arranged around the centrosomes as a focus. More recently F. R. Lillie ('12) in the egg of *Nereis* shows the repulsion of oil drops from the centrosome towards the egg membrane after fertilisation, while Meves ('12), in some excellent figures of the egg of *Parechinus*, shows that, during the development of the amphiaster the mitochondria are attracted towards it and collect in that zone which, by comparison with Chambers' work, one would imagine to be the very viscous zone. This arrangement agrees with that predicted above, where it was stated that heavier particles would collect in this zone.

In a work on mammalian oögenesis Cattaneo ('14) figures the arrangement of the Golgi apparatus during the growth of the oöcyte of a bat. In the youngest stages the apparatus is represented by a closely packed group of rods around the centrosome. As the oöcyte grows, the rods become scattered throughout the cytoplasm, while in the oldest stage, figured they are collected at the surface of the cell in a clearly defined peripheral layer.

Ludford and Gatenby ('21) in a recent paper describe the grouping of

the Golgi elements in various germ cells. In the young spermatogonia of the Rat the Golgi apparatus "appears as a compact mass of black rod-like structures embedded in the centrosphere." This mass is described as dividing into two as the result of the division of the centrosome. The two masses move apart to the opposite sides of the nucleus and in between them is formed the spindle. As the chromosomes become arranged on the equatorial plate the "Golgi rods break away from the archoplasm and become scattered in the cytoplasm." During telephase these rods are all gathered together again once more as a closely packed mass around the centrosome. Ludford suggests that the force which holds the rods around the centrosome during the early stages of mitosis becomes diverted at metaphase to draw apart the chromosomes. However, there need not be supposed any such selective action on the part of the centrosome if, as seems more feasible, it is assumed that the movements of these bodies to and from the centrosome are due to a periodic change in the relation of their density to that of the continuous phase of the cytoplasm.

Chambers (17) showed that if the nucleus of an *Echinarachnius* egg is separated from the aster, then as long as it is within the confines of the astral rays it will find its way back to the centre of the aster. He explains this as being due to the centripetal current flowing in the astral rays. As the amphiaster is formed while the centrosome is close up against the nucleus, the latter will be drawn into the spindle region and held there. When the chromosomes make their appearance from the nucleus they will therefore also be in the spindle region.

Lamb states that in metaphase the chromosomes will be repelled from the centrosomes. Owing to this repulsion they will collect at the equatorial plane but they will not then move outwards to the peripheral part of the equatorial plane for, though being repelled from the centrosomes, their induced oscillations causes them to attract one another.

If this is so an analogy can be drawn with the well known experiment of Mayer's floating magnets. A number of corks, through each of which is fixed a similar small rod magnet, are floated in a vessel containing water in such a way that all the magnets are pointing vertically upwards and all with the same pole uppermost, the north pole for instance. Since similar magnetic poles repel each other, these floating magnets will also repel each other and so collect at the sides of the vessel. If now a strong south pole is brought over the water on which the magnets are floating the latter, while still exerting a repellent action on each other, will be attracted towards that south pole and they will

now arrange themselves in equilibrium in a definite order. It is found that any number up to five arrange themselves at the corners of a regular figure. Thus three occupy the corners of an equilateral triangle, while five arrange themselves at the corners of a regular pentagon. If, however, there are six magnets they do not arrange themselves at the corners of a hexagon, but five take up positions at the corners of a pentagon while the sixth passes to the centre of the figure. A seventh magnet added to these six does not pass to the centre, but now six occupy the corners of a hexagon and one passes to the centre. In other words, they form an outer ring of six and an inner ring of one. With ten magnets there are formed an outer ring of eight and an inner ring of two and so on:

Total number of magnets	5	6	7	8	9	10	11	12	13	14	15	16	
Inner ring	...	...	0	1	1	1	2	3	3	3	4	5	5
Outer ring	...	...	5	5	6	7	8	8	9	10	10	10	11

It is obvious that if the chromosomes, when on the equatorial plate, are acted upon by two forces, one of repulsion from the centrosomes and another of attraction for each other, they are analogous to these floating magnets, and hence, if they are all of approximately the same size and if they are free to move, they should show an arrangement on the equatorial plate similar to that assumed by the floating magnets.

In such an arrangement it is obvious that any chromosome of the group can change places with any other chromosome of the group without disturbing the equilibrium, since they are of equal size, and it is presumed, mass. However, in a group in which one or more of the chromosomes differs in size from the rest, naturally such an interchange could not take place without leading to an unstable condition. Hence the equilibrium arrangement of such a group of different sized chromosomes would not conform to the same laws as those predicted for groups of chromosomes of the same size.

That the chromosomes are free to move on the equatorial plate can hardly be doubted. Microdissection work indicates that the cytoplasm in the equatorial zone is in a sol condition, and hence it is natural to suppose that the chromosomes would be free to move in such a medium.

It must be emphasised that at present, any view of an equatorial plate is usually obtained from sections of fixed materials, and naturally any slight distortions produced in fixation will lead to anomalous results. However, Fig. 2 shows examples of the arrangements of groups of chromosomes copied from various cytological works. They are chosen only

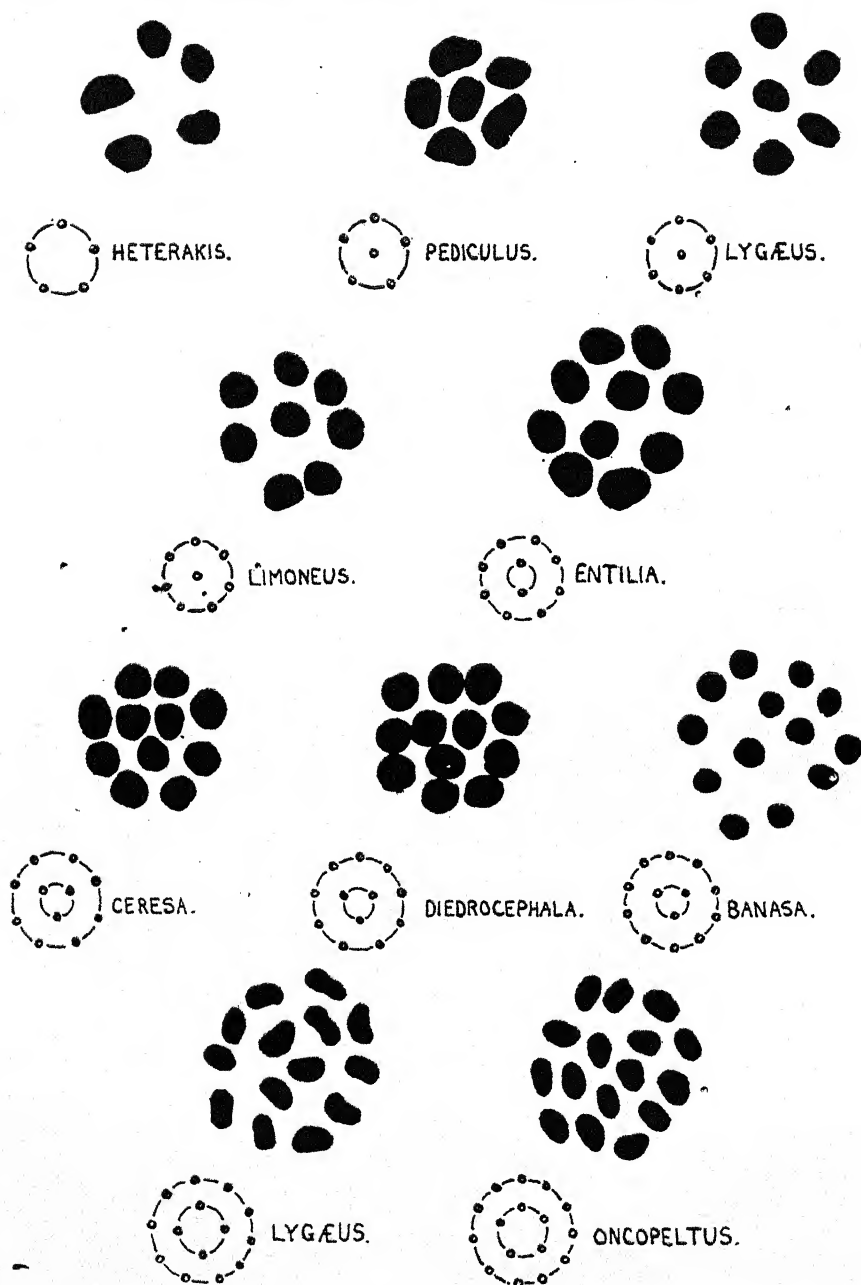


Fig. 2. Illustrating figures of equatorial plates, in each of which all the chromosomes are practically of the same shape and size. Under each plate is the name of the genus from which it is taken and in front of this name is figured the predicted arrangement of the chromosomes.

[*Heterakis*, Gulida, '11; *Pediculus*, Doncaster and Cannon, '20; *Lygaeus*, Wilson, '12; *Limoneus*, Stevens, '09; *Entilia*, *Ceresa*, *Diedrocephala*, Boring, '07; *Banasa*, Wilson, '05; *Lygaeus*, *Oncopeltus*, Wilson, '06.]

from examples where all the chromosomes appear to be of the same size and shape. The agreement with the predicted grouping is obvious<sup>1</sup>.

R. S. Lillie ('05) first pointed out that the arrangements of the chromosomes on an equatorial plate should coincide with those of Mayer's floating magnets. However, he based his views on the supposition that the chromosomes repel one another and are attracted at the same time by the protoplasm of the cell towards its centre. It is difficult to understand from this how it is possible for the chromosomes in a metaphase spindle to move towards the surface of the egg as they do in some cases of polar body formation and still remain in that spindle. The fact that the chromosomes remain in one plane, Lillie states, "remains to be accounted for."

More recently Doncaster ('20) has pointed out this resemblance and, moreover, he emphasised the fact that the coincidence between chromosome arrangement and that of the magnets is more marked when the chromosomes are short and of nearly uniform size. He did not suggest any explanation, but states that the fact "may have some bearing on the theories concerning the mechanism of nuclear division."

As was stated previously in discussing the formation of the aster under the influence of the centrosome, the particles lighter than the continuous phase of the protoplasm will be repelled from the centrosome and, since the egg is not limitless, there will be an accumulation of these lighter particles at its boundaries. That is to say, there will be formed a peripheral zone of lighter constituents at the egg membrane, that will not be free to move in a radial direction, and yet will still be acted upon by a force, repellent from the centrosome. Also since action and reaction are equal and opposite, this surface layer will repel the centrosome with an equal force. If this is indeed so, there will be certain phenomena which will naturally follow, and these will now be discussed.

In the first cases to be considered it will be assumed that the lighter material, after being repelled to the surface, remains collected there. The more general case, in which the lighter material is free to move in a tangential direction, will be considered later (see p. 68).

Bjerknes showed that the repellent force of an oscillating body for a neutral "lighter" body varies inversely as the 7th power of the distance between the two bodies. Thus, if the peripheral zone of an egg exerts

<sup>1</sup> Since writing the above, there has appeared in this Journal (Winge, *Journ. Gen.* Vol. xii. 1922, Plate XI, fig. 1) a figure of a polar plate of 23 chromosomes which are all practically of the same size and shape. They show very markedly the predicted grouping of an inner ring of two, a middle ring of eight and an outer ring of thirteen.

a repellent force on the centrosome, then, the nearer the surface is to the centrosome, the much greater will be that force. If an aster is placed centrally in an egg, the repellent force of the egg membrane will be the same in all directions. But, in the case of a mitotic spindle in a cleaving egg, each centrosome is excentric and hence the egg membrane on that side to which the centrosome is nearest exerts a greater force on that centrosome than does the diametrically opposite side of the egg membrane. Thus the two centrosomes are being forced towards each other by the surface layers of the egg. They will repel each other because of their own inherent oscillations. They will be repelled by the chromosomes on the equatorial plate, as has already been discussed. In the plane of the equatorial plate, all the lighter particles which have accumulated there will be in equilibrium and all these particles will be repelling the centrosomes. The chromosomes, however, will be by far the largest of these particles, and so the repellent action of the material in this plane will be chiefly due to the chromosomes. As a result then of Lamb's hypothesis, the length of a spindle in a dividing egg in metaphase will depend on (1) the repulsion of the centrosomes for each other, (2) the repulsion by the centrosomes of the chromosomes and other lighter material on the equatorial plate, and (3) the repulsion of the centrosomes by the peripheral zone of the egg. The first two factors tend to lengthen the spindle while the third tends to shorten it, and further, it is this last factor alone of the three which influences the position of the spindle in the cell. It is obvious that a spindle is not always repelled equally in all directions so that it moves towards the centre of the egg. Thus in polar body formation the spindle often passes from the centre to the surface. This will be discussed later (see p. 70).

This consideration of the factors determining the spindle length agrees well with certain findings of Conklin ('12), and of Herlant ('11) and Meek ('14). Meek ('14) states that there is a relation between metaphase spindle length and cell volume in *Forficula*. In the primary spermatocytes the nuclear material is of the same amount as in the spermatogonia, while the cytoplasm at the end of the growth period is much larger. That is, in the spermatogonia the peripheral layer of cytoplasm is nearer to the centrosomes than in the case of the primary spermatocytes. Correspondingly, the lengths of the metaphase spindles in these two types of cells vary in a ratio approximately the same as that between their volumes.

Conklin showed that, after the commencement of cleavage in the egg of *Crepidula*, the greater the number of cleavages that have taken



place, the smaller will be the spindle. Here, although there is, according to Conklin, an increase in the total amount of chromatin in the embryo, the amount in each cell in the form of chromosomes is diminishing with each successive cleavage, and this may cause the decrease in the spindle length.

Herlant showed that in di- and tri-spermic eggs, the spindle which forms around the fusion nucleus resulting from the copulation of the female pronucleus with one of the sperm heads is longer than those which form around the single sperm heads. He states "Il y a donc une relation déterminée entre la longueur d'axe d'une mitose et la masse nucléaire à diviser."

These last two phenomena, however, could be explained without assuming a repellent action of the egg membrane on the centrosomes, by the improbable assumption that the length of the spindle depends on the repellent action of the centrosome for the equatorial plates and the attraction, at metaphase, of the centrosomes for each other. In this case, however, the position of the spindle in the egg would show no spacial relation to the egg membrane. That there is a relation between the position of the spindle with reference to the egg membrane is shown by a recent experiment of Chambers ('19) on removing part of the cytoplasm of a segmenting egg. He states "a piece was cut from one pole of the amphiaster egg. The amphiaster in the remainder of the egg disappeared to reappear again in a new position, with the result that two equal sized blastomeres were formed." The new surface, that is, the surface of the cut, was nearer to the spindle, and thus repelled it more than did the opposite surface; and so the spindle was forced to the centre until it again took up a symmetrical position, with the result that two equal blastomeres were formed.

Conklin ('12), referring to the egg of *Crepidula*, states that, although by centrifugal force, the substance of the egg can easily be stratified into zones of material arranged according to their densities, yet the mitotic figure after the prophase, can be moved only with great difficulty. It seems from this that the chief factor which localises the spindle in the egg is the repulsion of the centrosomes, not by the cytoplasmic inclusions, but by the condensed peripheral zone of cytoplasm in which, as maintained above, there will be an accumulation of the disperse phase.

Perhaps Hertwig's law that the spindle axis, in general, tends to point towards the greatest protoplasmic mass can be explained in a similar way. The direction of greatest protoplasmic mass is, in other

words, that direction in which the cell wall is furthest off, and hence from this direction there will be the least repulsive force acting on the centrosomes. Thus the nearer peripheral zone will repel the spindle with a greater force until it comes to lie with its poles pointing towards the region of least repulsive force, that is, in the direction of greatest protoplasmic mass.

A phenomenon which, according to F. R. Lillie ('19), may be universal is that of the rotation of the sperm head during fertilisation. After the sperm head has penetrated the surface of the egg, its apex points towards the centre of the egg. At its outer part, that is, in the region of the middle piece of the spermatozoon, the sperm aster develops. As it develops it passes towards the centre and, synchronously, the sperm head pivots in such a manner that its base is directed towards the centre. Now Chambers ('17) has shown "that the sperm nucleus is held in a gel around the sphere and is dragged about by the aster." According to the ideas developed above, as the sperm aster develops it will be repelled towards the centre by the peripheral zone of the egg. The sperm head will not be acted upon by any such force, and so will remain where it is while the aster commences to move inwards. Thus the rotation of the sperm head will be due to the repellent force of the outer zones of the egg on the sperm aster and the inertia of the sperm head, combined with the fact that the sperm head is held by the gel of the aster.

The effect of the force acting between the centrosomes and the surface layers of the egg will now be considered in relation to that event of mitosis which has been termed "plasmadiaeresis," that is, the division of the protoplasm of a cell into two daughter cells as distinct from the division of the centrosomes and chromosomes.

That the shape of cells is due to the action of surface tension is expressed in Errera's law, which states that any group of cells in contact arrange themselves as if each were a soap bubble and devoid of mass. D'Arcy Thompson ('17) states "among the factors which determine the form of cells, whether solitary or arranged in contact with one another, this force of surface tension is certainly of great, and is probably of paramount importance."

Certain workers make the assumption that the pressure inside a cell is due to the surface tension at its surface. Thus D'Arcy Thompson ('17) calculates the curvature of the interface between a large and a small cell in contact, on this assumption. McClendon ('13) and Spek ('18) both calculate the theoretical value of the pressure inside an egg during

mitosis in terms of the surface tension of its surface, as if it were a fluid drop.

In recent years there have been published some very indefinite accounts of the actual physical state of the surface layers of a cleaving egg and these owe their lack of precision to the indiscriminate use of the word "gel." This has been emphasised and adequately discussed by Seifriz ('20) who is the first worker who, to avoid this confusion, gives in his own work, a list of the terms of viscosity which he uses in his description compared with the viscosity of certain well known solutions.

By the term "gel" one ordinarily understands a solid body, that is, one with infinite viscosity and showing elastic properties. Thus Mathews ('16) states that "gels are solids." Hatschek ('20) makes the general statement that a gel shows perfect elasticity within certain limits. But as Seifriz ('20) points out, actually, whether a colloid system is in a state of a sol or a gel depends upon its structure and not upon its viscosity. That there may be certain peculiar gels, that is, colloid systems showing the structure of a gel which at the same time exhibit a measurable viscosity, is undoubted, but since, in microdissection work, practically the only criterion as to the nature of the colloid system is its viscosity, it seems advisable to avoid the use of the word "gel" as much as possible and merely refer to the viscosity in terms of some standard scale as used by Seifriz.

Now a highly viscous fluid is not truly elastic—it may be resilient. Further, such a fluid would exhibit properties due to surface tension, while a solid which has infinite viscosity is unable to manifest its surface tension. Obviously, whether the surface of a dividing egg is fluid and so under the influence of surface tension, or whether it is solid and so under the influence of the tension in that solid is a matter of great importance as the two cases lead to totally different results. Thus, imagine an egg as a drop of fluid surrounded by a solid elastic membrane and suppose it were possible to inject into that egg an additional amount of fluid. This would stretch the egg membrane and so increase its tension, and this increased tension would increase the pressure inside the egg. On the other hand, suppose that the egg surface were fluid and so merely under the influence of its surface tension, then, if an additional amount of fluid were admitted into the egg a quite different state of affairs would arise. There would be no increase in tension in the limiting surface of the egg as the surface tension of the fluid *ceteris paribus* will remain constant, but there would be a decrease of pressure inside the egg, for the pressure inside a spherical drop of fluid is given by the

expression  $2T/R$  where  $T$  equals the surface tension of the fluid and  $R$  equals the radius of the drop. Thus if  $T$  remains constant and  $R$  is increased, the resulting pressure must decrease.

On *a priori* grounds it is difficult to imagine a sphere bounded by a solid membrane dividing into two parts, without this division leading to such inequalities in the tensions of the different regions of the surfaces of the two daughter drops as would make the spherical form of those daughter drops impossible. However from the works of Kite and Chambers on microdissection it is obvious that although they refer to the surface layer of an egg as being in a gel state they do not mean to imply that it consists of a solid but rather of a highly viscous fluid. Thus Kite ('13) used the term gel to designate the amorphous "semi-solid" state of living substance and this must also imply "semi-fluid." Chambers ('18) states that the fluid cytoplasm of an egg is enclosed in a "jelly like and highly viscous surface layer." Again Chambers ('17) emphasises the fact that "the gel state is never even visibly an inert solid" and again "living protoplasm, even in the gel state is a dynamic structure and never a static one." In a later paper ('19) he states that during the cleavage of an egg "two spheres of solidification grow at the expense of all but probably a small peripheral part of the fluid egg substance." Thus he considers the surface of a dividing egg as essentially fluid. Kite ('13) states that in the egg of *Asterias* small pieces of the peripheral portion can be drawn out into a thread and when freed the thread contracts "into a more or less rounded mass." Now if the egg surface had been a true elastic solid the protoplasm forming the thread would have contracted into its original form without leaving any trace. That it left a rounded protuberance indicates that it was essentially a viscous fluid.

Some experiments of Yatsu and Chambers on cutting away portions of cleaving eggs can be satisfactorily explained if the surface of the egg is assumed to be a liquid surface. Yatsu ('08) cut the cleaving egg of *Cerebratulus* unequally after it had formed a waist, in a plain parallel to the spindle axis. The larger portions contained the asters and the chromosomes. In the smaller piece which, naturally, contained no asters, the cleavage furrow deepened and cut the piece into two. The enucleated fragment can be considered approximately as a cylinder with a narrow middle portion forming a waist. Now the stability of a fluid cylinder depends upon the ratio between its length and its diameter. If this ratio becomes too high the cylinder becomes unstable and divides into two at its middle. Hence if the surface of the egg of *Cerebratulus* is

fluid, the obvious explanation of the division of this enucleated fragment is that its length was too great compared with its breadth for it to be stable. Similarly Chambers ('19) cut the egg of *Asterias* just as it was beginning to cleave in a plane oblique to its spindle axis, so that it was divided into two pieces, each of which contained an aster. Sometimes the cleavage furrow persisted, and a small enucleate fragment was budded off from each nucleated fragment, while at other times the cleavage furrow disappeared and each piece then appeared like a normal blastomere. These wedge-shaped fragments cannot be so strictly compared to a fluid cylinder but obviously their stability will depend on similar conditions. There seems to be no other feasible explanation of the division of these enucleate fragments.

Seifriz ('21) in his most recent work on protoplasmic membranes states that he agrees with Berczeller ('17) in looking on the plasma-membrane not as "a skin," which Berczeller defines as a rigid structure "but as a highly viscous layer of modified protoplasm which may at times become quite fluid." He is careful to indicate that he is dealing with the surface of naked protoplasm only.

From the above discussion it must be inferred that the surface of the cytoplasm of a dividing egg, at least during the last half of mitosis is essentially a fluid structure. Whether sol or gel is not of great importance here, the important fact to be deduced is that, since the surface of the dividing egg is fluid, the pressure inside that egg is determined by the general equation connecting the pressure inside a fluid drop with its surface tension and its curvature  $P = T(1/R_1 + 1/R_2)$  where  $P$  = pressure inside the drop,  $T$  = surface tension of the fluid and  $R_1$  and  $R_2$  = the maximum and minimum radii of curvature at any point. These two radii are always in planes at right angles to one another.

The initiation of plasmodiaeresis must be coincident with a relative increase in surface tension in the equatorial zone. Robertson's ('13) assertion that the division of a cell is a resultant of a relative decrease in the surface tension in the equatorial zone has been adequately disproved by McClendon ('13)<sup>1</sup>.

Chambers ('19) does not mention any such local change in surface tension in the egg surface. He explains plasmodiaeresis as being due to the growth within the cell of two solid masses of material at the expense

<sup>1</sup> In a recent paper (*Q.J.M.S.* Vol. LXVI. 1922), Gray has maintained that cell division can be readily explained without postulating any differential surface tension at the poles or equator of the cell. His views are refuted by the present author in *Nature*, Vol. cx. p. 2753, 1922.

of the surrounding cytoplasm. That is at the expense of the peripheral cytoplasm which he further explains is fluid. It is easy to show, that under these conditions, at that stage at which a waist is formed during the division of an egg, that egg would be in an unstable condition and all the peripheral fluid would tend to collect at the equator and so obliterate the furrow as soon as it was formed. Actually, however, this stage could never be reached if plasmadiaeresis proceeded according to Chambers' ideas, for, as soon as the two solid spheres in the fluid spherical egg had grown so that their combined diameters equalled the diameter of the fluid drop in which they were situated, then, any further growth would stretch the diameter of the drop in one direction and it would naturally decrease it in another direction at right angles to this, as Chambers points out. That is, it would lead to some form of ellipsoidal drop, and such a drop is unstable if the surface tension is uniform throughout its surface. Its fluid portion would transform immediately into a spherical drop surrounding one of the spheres of solidification. If there is assumed to be an equatorial zone of relatively higher surface tension in the dividing egg, then Chambers' ('19) view is theoretically possible. But, as will be shown immediately, without certain other forces coming into play it is practically impossible.

That an increasing surface tension in an equatorial zone will cause a waist to develop can easily be deduced from the general equation quoted above and will not be discussed further here.

Imagine a fluid drop in which a waist has commenced to form which would tend to divide it into two equal daughter drops. Each half of the drop can be considered as a part of a sphere. Now the pressure inside either half of the cleaving drop will be  $2T/R$  where  $R$  is the radius of curvature of the spherical surface of either half. If, through the action of some external force one half becomes smaller than the other half, the pressure of the smaller one will immediately become greater than that in the larger one, since its radius of curvature has become smaller. Since fluid flows from regions of high pressure to regions of low pressure it naturally follows that fluid will flow from the smaller half into the larger. This, however, will further diminish the radius of the smaller half, increase its internal pressure, and so cause the flow of fluid to increase. This will obviously proceed until all the smaller drop has flowed into the larger one. In other words the division of a fluid drop under the sole influence of an increasing surface tension in an equatorial band, is only theoretically possible.

In a cleaving egg there is a similar state of affairs—when once the

division furrow has commenced, any slight disturbance due to external forces, such as currents, waves, etc., could compress or expand either half of the dividing egg. But, as is shown above, when once this deformation started, it would proceed until one half of the egg had absorbed the fluid portion of the other half. An egg which has commenced to divide under such conditions is therefore in unstable equilibrium and so division of an egg, merely by the influence of this force, into two equal portions is not actually possible.

It may be pointed out that Robertson ('13) and Spek ('18) have been able to cause a fluid drop to divide into two, solely through the action of a varied equatorial surface tension, but in all these cases large drops were used in which the internal pressure is not great, and also, the division was comparatively rapid. The possibility of division is also due to the viscosity of the fluid used, which would tend to prevent the flow of one drop into the other. In a dividing egg, however, the division is, in comparison, extremely slow, and so the viscosity, despite its large value, as a factor tending to inhibit the flow of one drop into the other, would not produce a marked effect. In any case equal division of the egg is, under these conditions only theoretically possible.

It must be emphasised that in such a small body as, for example, the egg of a sea-urchin, with its fluid surface, the internal pressure must be very great. And, also, in the cleaving egg any small difference between the radii of the prospective blastomeres would lead to a large difference of internal pressure between them. Cases of unequal cleavage in which there is "polar lobe" formation are merely special cases of equal cleavage, but in those cases of unequal cleavage where there is no polar lobe, then, when the cleavage furrow is forming, the action of surface tension will tend to set up a large difference of pressure between the two blastomeres. This difference of pressure would quickly cause the fluid contents of the smaller sphere to flow with increasing speed into the other unless there were some counteracting force.

Now this counteracting force will be supplied by the repellent action of the centrosomes on the surface layers of the egg. Bjerknes ('00) states that a lighter body will be repelled from an oscillating body with a force which varies as  $1/R^7$ . This means that the intensity of the force with which a centrosome situated at the centre of a spherical blastomere will repel the surface of the blastomere will also vary as  $1/R^7$  and hence can be represented by  $K/R^7$ .

Ignoring for the time being the action of surface tension, imagine as before, a cleaving egg in which the waist has just commenced to form,

and suppose that through some external force, one half of the egg is diminished in size. Now, in this smaller half, the repellent action of the centrosome for its surface will increase, since its radius has diminished, and, further, this force is in an outward direction. That is, it will tend to cause the smaller half to expand again to its original size. In other words, if, through external causes, the shape of a dividing egg is disturbed, this repellent force on the surface layers tends to restore the cleaving egg to its original condition and so it will counteract the opposite effect of the surface tension which tends to increase any deformation. Surface tension tends to render the cleaving egg unstable while the centrosomal force tends to stabilise it.

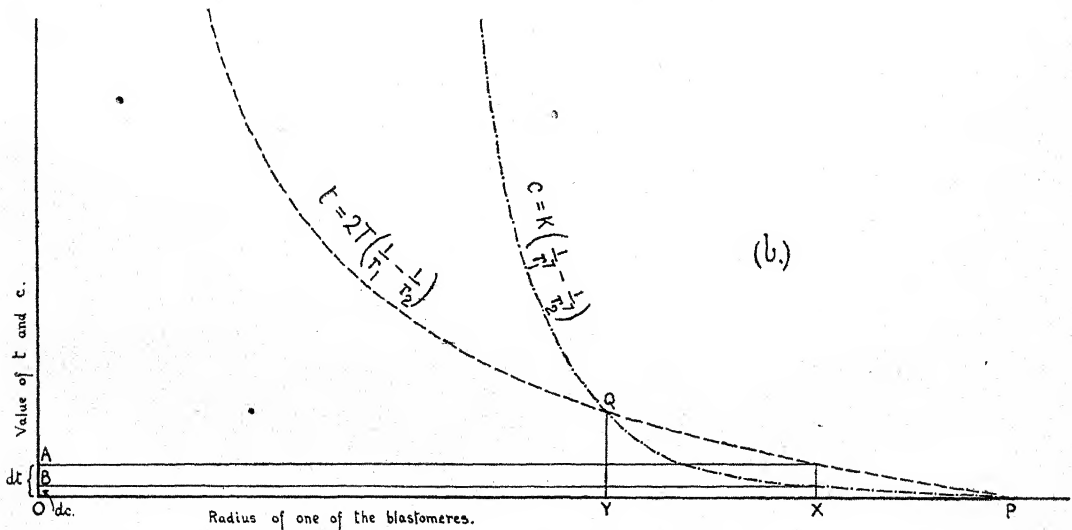
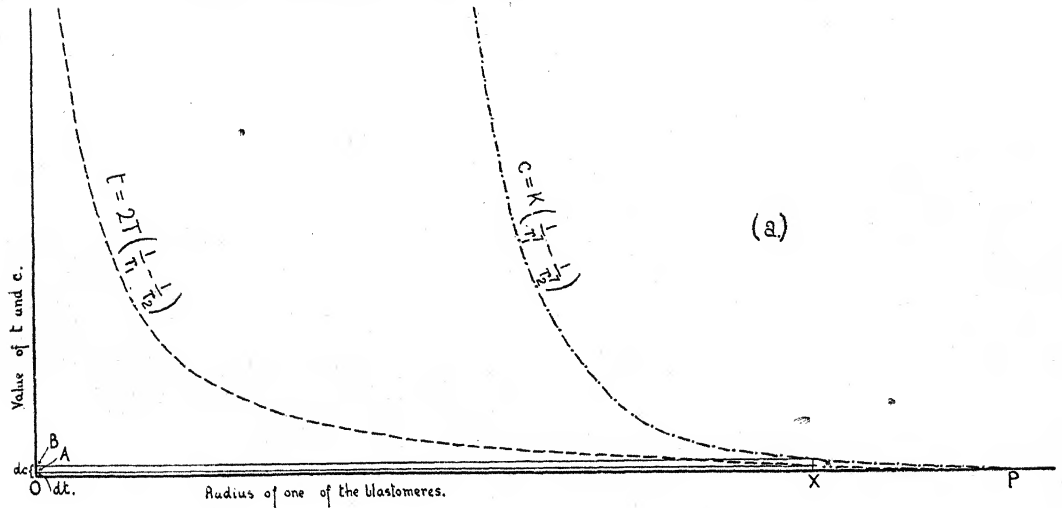
The two halves of the dividing egg have been considered as parts of spheres. It will not be far wrong, if it is assumed that, during cleavage if  $R_a$  and  $R_b$  are the radii of curvature of each potential blastomere, then, since the sum of the volumes of the two blastomeres is constant  $(R_a)^3 + (R_b)^3 = K$ , this  $K$  being a measure of the volume of the egg before cleavage. If this is assumed, it is possible to obtain the series of values connecting  $R_a$  and  $R_b$ .

Now the force due to the surface tension which tends to cause the protoplasm to flow from the temporarily smaller blastomere into the larger will be given by  $t = 2T/R_a - 2T/R_b$ ,  $R_a$  denoting the radius of the smaller of the blastomeres. This will be counteracted by the resultant of the centrosomal repulsion on the surface of the blastomeres and this will be given by  $c = K/R_a^2 - K/R_b^2$ . Obviously equilibrium can only obtain when  $t = c$ .

It is not possible to obtain the actual values of  $t$  and  $c$  since the absolute values of  $K$  and  $T$  are not known, but if one plots the values of  $1/R_a - 1/R_b$  and of  $1/R_a^2 - 1/R_b^2$  one will obtain curves of the same form as if the actual values of  $t$  and  $c$  had been plotted, and hence, deductions can be made from these curves which will apply equally well to the actual relations of the values of  $t$  and  $c$ . If this is done it is seen, as might have been deduced from the equations themselves, that the curves are of different shapes (Figs. 3a and 3b). In these graphs both the curves cross at the point  $P$ . This has been taken as the point when the two blastomeres are of equal dimensions and so  $R_a = R_b = OP$ . Here  $t$  and  $c$  are equal as they are both zero and thus the egg is in equilibrium. Whether this equilibrium is stable or not can be deduced from the curves. Suppose, as before, that one blastomere becomes smaller than the other. Its radius will be represented by a length  $OX$  where  $X$  is some point nearer to  $O$  than  $P$ . Let this diminution be represented



by  $dR$ . For this small change  $dR$ , there will be corresponding changes in  $t$  and  $c$  which can be represented by  $dt$  and  $dc$ , and in the curves these will be represented by the lines  $OA$  and  $OB$ . In Fig. 3a,  $dc$  is greater than



Figs. 3a and 3b.

$dt$  and since the force  $c$  tends to produce stable equilibrium, the cleaving egg will return to its former condition. Thus the point  $P$  in Fig. 3a represents a point of stable equilibrium and so equal division of the egg is possible.

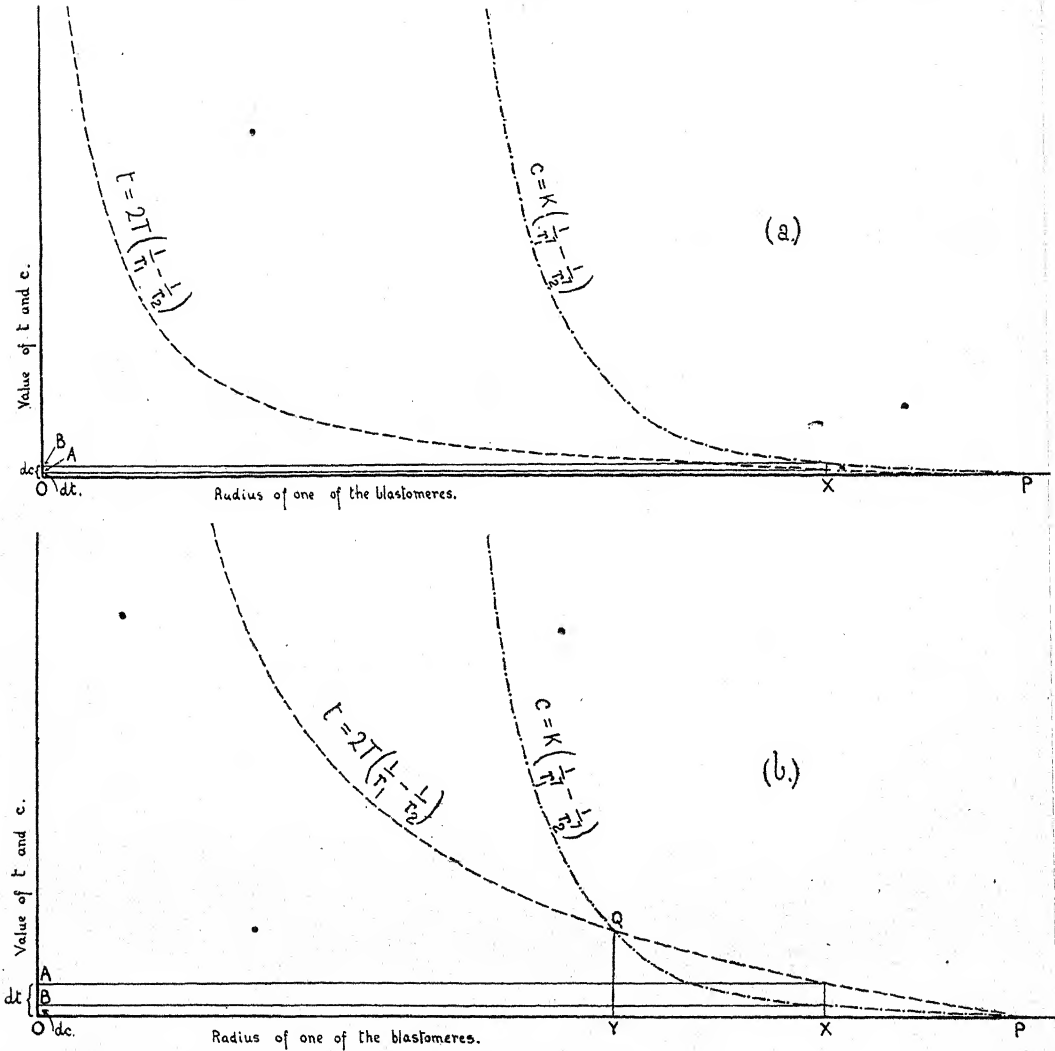
and suppose that through some external force, one half of the egg is diminished in size. Now, in this smaller half, the repellent action of the centrosome for its surface will increase, since its radius has diminished, and, further, this force is in an outward direction. That is, it will tend to cause the smaller half to expand again to its original size. In other words, if, through external causes, the shape of a dividing egg is disturbed, this repellent force on the surface layers tends to restore the cleaving egg to its original condition and so it will counteract the opposite effect of the surface tension which tends to increase any deformation. Surface tension tends to render the cleaving egg unstable while the centrosomal force tends to stabilise it.

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Figs. 3a and 3b.

$dt$  and since the force  $c$  tends to produce stable equilibrium, the cleaving egg will return to its former condition. Thus the point P in Fig. 3a represents a point of stable equilibrium and so equal division of the egg is possible.

It has been emphasised previously that the actual loci of the curves will depend on the absolute value of the surface tension and the other constants concerned, but the shapes of the curves will remain the same irrespective of the values of those constants. Since the two curves are of different shapes they can never coincide, but it is legitimate to assume that, in some cases, the real foci of the curves will be such that, although they cross at the point  $P$ , where the size of the two blastomeres is the same, they will also cut at another point  $Q$  (Fig. 3*b*). After crossing at  $Q$  they will meet only again at infinity.

In this case as before, a small change  $dR$  leans to a corresponding change  $dc$  and  $dt$ , but here  $dt$  is greater than  $dc$ . This means that, for a small change in the size of one of the blastomeres, the effect of the surface tension which tends to increase that disturbance is greater than the centrosomal force,  $dc$ , which tends to restore equilibrium. Thus here, at the point  $X$ , the cleaving egg is not in equilibrium and so the smaller blastomere will diminish further. This will proceed until the radius of the smaller blastomere is represented by  $OY$ , where  $Y$  is vertically below the point  $Q$ , where the two curves cross. Now at this point  $t = c = QY$ , and hence the cleaving egg must be in equilibrium. It will easily be seen that the point  $Q$  in Fig. 3*b* is similar to the point  $P$  in Fig. 3*a*, that is, it is a point which represents stable equilibrium of the cleaving egg in which the blastomeres are of unequal size. In Fig. 3*b* the point  $P$  represents equilibrium but it is unstable. Hence it can be deduced from this curve, that plasmadiaeresis is possible, under those conditions from which the curve is derived, when the sizes of the two blastomeres are different.

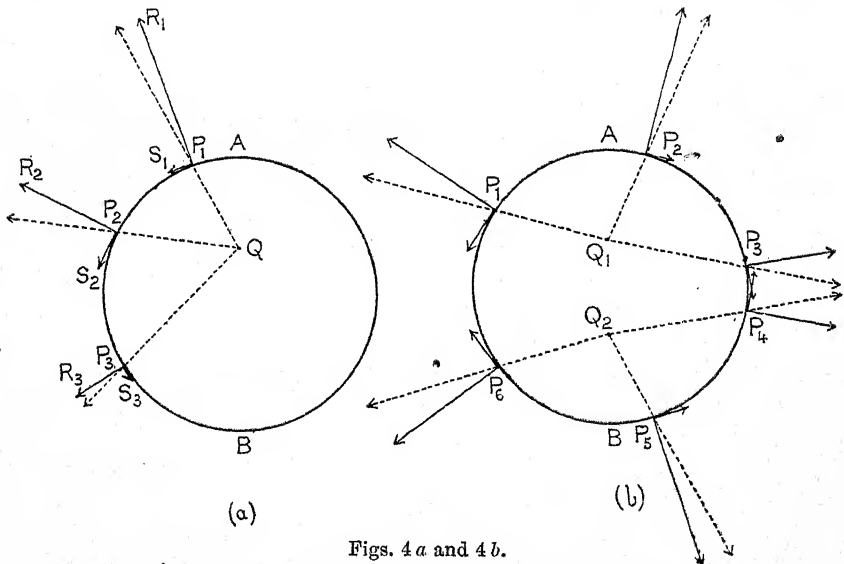
Thus it has been argued that experimental evidence indicates that the surface of a cleaving egg is essentially fluid. From this it has been deduced that cleavage cannot be brought about through the sole agency of surface tension, and hence that there must be some force which counteracts the disturbing force of surface tension. It is maintained that the centrosomal force postulated in Lamb's hypothesis will result in such a stabilising action, and further, that this hypothesis permits the possibility, not only of equal, but also of unequal cleavage.

In considering the repellent action of the centrosome on the surface layers up to now, it has been assumed that the lighter material, once it collects at the surface, remains there. The alternative case in which the lighter material is free to move in a tangential direction remains to be considered.

In Fig. 4*a* the point  $Q$  within the circle  $AP_1P_2B$  represents an

eccentrically placed centrosome of an aster in a spherical egg, the cytoplasm of which is theoretically uniform. The repellent forces due to the centrosome acting on particles  $P_1 P_2 \dots$  which have collected at the surface will act along the lines  $QP_1, QP_2, \dots$ . These may be resolved into forces normal to the surface of the sphere  $P_1 R_1, P_2 R_2, \dots$  and forces tangential to the surface  $P_1 S_1, P_2 S_2$ . The former components will merely tend to collect the particles at the surface but the other component will always act in a tangential direction from  $A$  to  $B$  and thus tends to drive the lighter particles from  $A$  to  $B$ .

If now the fluid forming the peripheral layers of the sphere has a comparatively low viscosity, the particles, once they have reached the



Figs. 4 a and 4 b.

surface, will not experience much resistance to their motion resulting from the tangential force acting on them, and so will move towards the pole  $B$  and collect there. If this movement from  $A$  to  $B$  has proceeded long enough the accumulation of the lighter material at  $B$  will become so great that the repulsive force of the surface layers at  $B$ , acting on the centrosome  $Q$ , will be greater than the force similarly exerted by  $A$ , and hence  $Q$  will move towards  $A$ . As it moves, however, the tangential component of the centrosomal force becomes comparatively greater than the radial component, and so the movement of the lighter materials from  $A$  to  $B$  will be accentuated and thus the centrosome will continue toward  $A$  until it reaches the surface.

If now, instead of the single aster, there is a spindle between the two centrosomes  $Q_1$  and  $Q_2$  symmetrically placed with regard to the egg, Fig. 4b, it is obvious that they will be antagonistic in their effects on the surface particles, and hence the lighter material will tend to accumulate in the equatorial region between the two centrosomes. But if the pair of centrosomes is slightly displaced towards  $A$ , then the resultant of the two centrosomal forces will be a balance in favour of the collection of lighter material at  $B$ ; *i.e.* the result can be considered as the sum of the effects of two independent centrosomes. Thus a spindle between two centrosomes in a diameter of the egg will act as a single centrosome, and thus would move towards the surface under the same conditions.

Further, if there is a spindle placed anywhere in the egg so that one centrosome is nearer the surface than the other, then that centrosome which is nearest the surface will tend to pass towards the surface more quickly and with a greater acceleration at any instant than will the other. They will both move towards the surface but the slower speed of the centrosome furthest from the surface will cause it to lag behind and this will result in the spindle, as it moves towards the surface, setting itself perpendicular to that surface. Actually, in an egg, this effect may be obscured by many things; thus the presence of a sperm aster would give rise to a different set of results. But, near to the surface, one would expect the effect to be most pronounced, and this is certainly the case in the formation of polar bodies where the spindle always ultimately comes to lie perpendicular to the egg surface.

In the cases considered above it is assumed that the fluid contents of the egg are comparatively non-viscous. If, however, the fluid, is, on the contrary, viscous, the resistance offered to the movement of lighter particles by the surrounding fluid may be greater than the force tending to collect them at one of the poles of the egg. If now a centrosome is displaced from a central position, there will now be comparatively no displacement of lighter material towards one pole of the egg; and so the force of repulsion exerted by the nearer surface will be greater than that of the opposite surface, and thus the centrosome will be forced back to its central position. Obviously a similar result applies to a spindle in like condition.

Thus to summarise, it may be stated that an aster or a spindle placed centrally in a spherical egg is in stable or unstable equilibrium according as the viscosity of the fluid surface layers of the egg is comparatively high or low. Accordingly the movement of an aster or an amphiaster in

an egg will depend, among other things, on the viscosity of the surrounding cytoplasm. With more viscous cytoplasm the centrosome will tend to pass to the centre while with more fluid cytoplasm it will pass from the centre to the surface.

Not much work has been published on the variations of viscosity during fertilisation, but Heilbrunn, working on *Arbacia* ('15 and '20) and on *Cumingia* ('21), and Seifriz ('20), using the eggs of *Echinarachnius*, have both discovered definite relations between the viscosity of the egg and the various events of fertilisation. In the case of *Arbacia* and *Echinarachnius* one would expect, from the uniform nature of the contents of the egg, that the above theoretical considerations would apply most closely. In these urchin eggs, polar body formation is completed before the entry of the spermatozoon. The sperm head directly after penetrating the egg membrane develops an aster about its hind end. This aster then passes to the centre of the egg. Hence it can be predicted that this process is associated with increased viscosity, at least in the peripheral part of the egg. Heilbrunn ('15) states that in the ripe egg of *Arbacia* the protoplasm is comparatively fluid, and that fertilisation is associated with increased viscosity.

In *Cumingia*, where maturation takes place after the entry of the spermatozoon; he showed that the passage of the first polar spindle towards the surface takes place when the egg-contents are comparatively non-viscous. He plotted a curve indicating the values of the viscosity during the period after fertilisation until the completion of the first cleavage. During the latter part of the mitosis, the curve rises, indicating a high value of viscosity, and falls again before the next mitosis. However, in between the high value of the viscosity during the second polar body mitosis and the low value preceding the first cleavage mitosis there is a very striking rise in the curve, indicating an increase in viscosity just at that time when the pronuclei pass towards the centre, which is in close agreement with the idea that the passage of an active centrosome towards the centre of the egg is associated with increased viscosity of the cytoplasm.

Heilbrunn's method of experiment was to centrifuge the eggs and note whether the contents of the eggs separated out into layers or whether the viscosity was large enough to overcome the disturbing centrifugal force. This method, of course, can give us no indication of any regional variation of viscosity. However, Seifriz ('20), by the microdissection method, has been able to determine local variations of viscosity in the egg of *Echinarachnius* after fertilisation. He states that the

viscosity of the unfertilised egg is similar to that of glycerine, and is practically uniform throughout the egg, but, with the first appearance of the sperm aster, there is an increase in the viscosity of the peripheral cytoplasm. By the term peripheral cytoplasm he does not mean the cell membrane, but a broad outer zone of cytoplasm. This conforms exactly with the theoretical results depicted above.

It has been pointed out already that the activity of a pair of centrosomes symmetrically placed in an egg will lead to an accumulation of lighter material in the equatorial zone. The presence of surface currents from the polar regions to the equatorial zone has been described in the cleaving egg of *Ascaris* by Spek ('18).

It is possible that this tendency for the lighter material to collect at the equator of an egg may sometimes be causally connected with an increase in surface tension and the consequent appearance of a waist in this region which is always a precursor of plasmodiaeresis. In this case, as the spherical egg divides into two smaller spheres, it tends to bring the surface of the incipient spheres normal to the centrosomal force in those spheres, and hence tends to minimise the force which might otherwise drive the two centrosomes to one of the poles.

The arrangement of the chromosomes on the metaphase plate has been discussed already. Their movement from the equator towards the poles is explained by Lamb, as was pointed out in the introduction to this paper, as being due to their varying density. Prenant ('10) criticises this by saying that any assumption as to changes of density of chromosomes is quite gratuitous and unsupported by any fact. But, whether periodic changes in density of chromosomes are probable or not, there is another obvious explanation. That is, that the density of the continuous medium in which the chromosomes are suspended, changes periodically. This could lead, on Lamb's hypothesis, to the same results as if the density of the chromosomes themselves changed periodically. It is not their absolute density, but the relative density of the chromosomes and the surrounding fluid which determines the sign of the force acting upon them. This suggestion cannot surely be termed gratuitous. There are many facts which indicate that most probably the density of the continuous phase of the cytoplasm changes periodically during the cell cycle. The mere fact that during mitosis the evolution of carbon dioxide is at a maximum strongly suggests that there are corresponding changes of densities in the fluid contents of the egg. In fact it is difficult to conceive of the cyclic changes that take place during mitosis, not being correlated with changes in density of the various fluid contents of the cell.



In the foregoing pages I have collected together all the theoretical results that appear to me to follow necessarily if we accept Lamb's hypothesis of the mechanism of mitosis. They may be summarised briefly as follows:

(a) The arrangements of the contents of a cell would depend *inter alia* on the activity of the centrosome, and also on their densities, relative to that of the continuous phase of the surrounding cytoplasm. This would lead to an explanation of

(b) The constitution of the aster and amphiaster as described by microdissection workers.

(c) It would account for the arrangement of the chromosomes on the polar plate of dividing cells in those forms in which the chromosomes are all of practically the same size and shape.

(d) The periodic movements of the cytoplasmic inclusions to and from the centrosomes might also be accounted for.

As a result of (a) there should be, during a period of activity of the centrosomes,

(e) An accumulation of "lighter" materials in the peripheral zones of the cell. This will lead to a mutual repulsion between the centrosome and the outer layer of the cell, and if this zone is comparatively viscous will result in

• (f) Both equal and unequal cell division being possible.

(g) The rotation of the sperm head during fertilisation would be explained.

(h) It would account for certain relations found between the spindle length and cell size.

(i) It would explain the movement of the sperm aster towards the centre of the egg during fertilisation. On the other hand, if the peripheral zone of the egg is comparatively non-viscous, there would follow an explanation of

(j) The movement of the spindle towards the surface of the egg as in polar body formation.

(k) And also of the fact that such a spindle always ultimately sets itself perpendicular to the egg surface.

The assumption that I have made in order to arrive at these conclusions is that the cytoplasm, at least in the neighbourhood of the centrosome, is an emulsoid colloid in the sol state. That is, I have assumed that there is one definite continuous phase. Recent work of Seifriz (21) may indicate that the surface layers of an egg, despite their fluidity, are in a gel state, in which case it is probably meaningless to talk of one phase being continuous and the others as being disperse. But

in the central parts of the cell, all microdissection workers seem to agree that the cytoplasm is in the sol condition, and it is in the central portion that the centrosome is ordinarily to be found.

As I pointed out earlier in this paper, there is no need to consider cytoplasm as being essentially a two-phase emulsion. Protoplasm may be, as Seifriz ('20) states, "a multi-phase system, emulsion within emulsion." All that is necessary for the results I have deduced is that it shall be in the sol condition and so there will be a definite continuous phase. Then, if the disperse phase is, on the whole, lighter than the continuous phase, a feasible explanation can be given of the structure of the astral figure as described by Chambers ('17). If, on the other hand, the disperse phase is on the whole heavier, then there would be no aster formed, and possibly no apparent activity, as there would be greater influx towards the centrosome than efflux from it, and so the disperse phase would be merely crowded on the centrosome. This would not prevent the gradual accumulation of the lighter materials at the surface, and so there would still exist a mutual repulsion between the centrosomes and the peripheral zones. Thus plasmadiaeresis would be quite possible without any aster formation. At the same time it would allow for the possibility that in some cases, as seems probable in certain leucocytes and epithelial cells, the centrosome may be in a continual state of activity without there being any astral radiations.

Prenant's criticism ('10) of Lamb's hypothesis I have discussed already. The only other important criticism is that of Hartog ('13). He negatives the idea of a hydrodynamic field due to oscillating bodies on the grounds that such fields are not possible in the heterogeneous and viscid contents of the cell on account of damping action. This is a very general statement and, I think, must surely depend, not only on the nature of the medium in which the bodies are oscillating, but also upon the source of energy of the oscillating bodies. This again will be dependent on the constitution of the oscillating bodies themselves.

Hartog states further that the extension of the rays into the cytoplasm is the sure proof that mitosis cannot be due to such a hydrodynamic field. I have tried to show that the form of the aster will be due to the passage outwards of the disperse phase and the passage inwards of the continuous phase, the latter forming channels leading towards the centrosome which correspond to the astral rays. It is obvious that, on this explanation, as the disperse phases pass outwards, so the astral rays will grow in length. Hartog ('14) however considered that the existence of twisted spindles formed "striking refutation of those

views that regard achromatin fibres as mere stream lines"—a view that could hardly be put forward nowadays in the light of recent microdissection work.

Further, according to the explanation I have put forward, the centrosome is oscillating in the continuous phase of the cytoplasm, a substance which is comparatively very fluid and not viscid as Hartog suggests. According to the description of Seifriz ('20) the fluid of the centrosphere is not much more viscous than water.

Lamb put forward his hypothesis as an explanation of the "mechanics of mitosis." Such a hypothesis should take into account all the various events which collectively constitute mitosis. Lamb's hypothesis deals rather with the nature of centrosomal force only, and hence the title of the present paper. Such a hypothesis should conform to all those events in which a centrosome is directly concerned, and among these must be included fertilisation. I have already considered the conditions of movement of a centrosome in a cell and I think that those considerations together with some of the results of microdissection are adequate to explain the meeting of the pronuclei and their passage towards the centre of the egg during fertilisation. D'Arcy Thompson ('17) says that the meeting of the pronuclei and their passage towards the centre of the egg "must be due (as Whitman pointed out long ago) to a force of attraction acting between the two bodies and another force acting upon one or both in the direction of the centre of the cell." With regard to the attraction for each other F. R. Lillie ('19) recently said that "there is no basis in fact beyond the meeting of the nuclei which can equally well be explained on other more reasonable and less mystical grounds," namely that "as both sperm nucleus and the egg nucleus are in physiological relations to the same mass of cytoplasm which is preparing to divide, they must reach the same position of equilibrium within the cell and hence of necessity meet." I cannot see that this explanation is any more reasonable until the mystical term "physiological relations" is defined. One might reasonably ask why do not all the sperm heads in polyspermic frog's egg meet the female pronucleus at the centre of the egg. Further Brachet ('17) has shown that in polyspermic eggs of the frog where more than one hundred sperm heads enter, the sperm heads do attract one another, provided that they have not yet developed astral radiations. He also states that one condition for the union of the two pronuclei is that one, at least, shall be deprived of an active centrosome. The explanation of the copulation of the pronuclei that I give here is that, as the sperm nucleus develops its aster,

its centrosomal force collects material at the surface of the egg which exerts a repellent action on the centrosome, and so forces it to a centre of equilibrium in the egg. The sperm nucleus is held in the highly viscous zone of the aster and so dragged towards this centre. The female pronucleus, which may or may not be attracted by the sperm nucleus, is dragged to the centre of the sperm aster, and so meets the sperm nucleus, by the centripetal currents in the astral rays as was described by Chambers ('17). The female pronucleus possibly is neither attracted nor repelled by the centrosome but more probably it is comparatively so large and so lacking in rigidity that any force of repulsion or attraction would be masked by viscous friction and so the pronucleus would be passively drawn into the sperm aster.

As to the nature of a centrosome Lamb makes no suggestion,—he merely states that the assumption of its being an oscillating body is "not even improbable." The present paper deals only with the force emanating from the centrosome. The question of its actual constitution is a totally different question and is, I think, one which cannot be profitably discussed in the present state of our knowledge<sup>1</sup>. However, Brachet ('17) states that the essential for the formation of an energid is not the centrosome—"C'est non pas le centrosome mais l'état physique nouveau du cytoplasme de l'œuf..." and further "nous ne voulons pas dire que le centrosome n'est rien...mais plutôt qu'il ne peut jouer son rôle qu'à la condition d'un état déterminé du cytoplasme soit réalisé. Peut-être le centrosome est-il toujours actif, mais le cytoplasme ne peut-il répondre à l'attraction (?) exercée sur lui, que périodiquement suivant rythme en rapport avec son métabolisme." This, I think, agrees completely with the deductions I have made from Lamb's hypothesis. The visible activity of the centrosome, that is, its power of forming an energid, will depend on the physical state of the cytoplasm, this physical state being determined by the relative densities of its constituents. The apparent periodicity of the activity of the centrosome will be a result of the cyclic changes in the constitution of the cell cytoplasm.

<sup>1</sup> It has been suggested to me that a case analogous to the hypothetical oscillation of the centrosome is to be found in the explanation sometimes put forward that ascribes the fluorescence of certain substances to a periodic tautomeric change in the molecules of those substances. Any activity involving periodic rearrangement of the atoms constituting the centrosome molecules might entail a corresponding variation in the field of force surrounding the centrosome. Such a variation might lead to volume changes sufficient to cause the oscillation supposed.

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# THE INHERITANCE OF GLUME-LENGTH IN A WHEAT CROSS (*continued*<sup>1</sup>).

By F. L. ENGLEADOW

(*Plant Breeding Institute, Cambridge*).

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## § I. *Introduction.*

The investigation here described was carried out in continuation of an earlier one [Engledow (1)] a brief résumé of which is necessary. To ascertain the precise mode of inheritance of a "measurable" plant character two reliable pure lines of wheat were crossed and measurements of glume-length made in the parental and the successive hybrid generations. For the parents the average glume-lengths were about 12.0 and 31.0 mm. respectively. One glume per ear was measured, the unit being 1.0 mm.: the satisfactoriness of this convention was tested by separate preliminary observations. Some sterility was encountered but its incidence in all the populations appeared to be fortuitous.

For easy reference the following symbols were employed, and will again be used:

*P* implies Polish (the one parent) or of Polish type.

*K* implies Kubanka (the other parent) or of Kubanka type.

*I* implies the heterozygote form of the hybrid generations and which, as later appears, is roughly speaking intermediate between *P* and *K*.

<sup>1</sup> For the earlier investigation see Bibliography (1).

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$F_0.P$  designates a population of pure "parental" Polish type.

$F_0.M_P$  denotes the mean glume-length of an  $F_0.P$  population.

$F_0.\sigma_P$  denotes the standard deviation of the glume-length frequency-distribution of such a population.

To cope with the seasonal fluctuation of mean glume-lengths,  $F_0.P$  and  $F_0.K$  were grown every year throughout the investigation.

The  $F_1$  plants were in appearance excellent "blends" of the parent forms and the value of  $F_1.M_I$  was about 16.0–18.0 mm. For  $F_2$  the frequency-distribution of glume-length was very clearly trimodal and its form suggested simple segregation on the 1:2:1 basis. Of the three types which might be presumed to constitute the  $F_2$  none, however, could be exactly like  $F_0.P$  for the *upper limit* of the  $F_2$  distribution of glume-length was at 30.5 mm. whereas the *mean* of  $F_0.P$  was at 30.84 mm. Nevertheless, to the eye, the  $F_2$  was constituted by three types, one absolutely like  $F_0.K$ , one apparently "intermediate" between the parents, and ~~and~~ absolutely like  $F_0.P$  though never attaining a glume-length greater than 30.5 mm. [In  $F_0.P$  glume-lengths up to 40.5 mm. occurred.] It seemed, in fact, that in  $F_2$  the " $P$ " type was represented by a population of plants whose mean and fluctuation-ranges for glume-length had been "shifted" down to a considerably lower value. By an empirical resolution of the  $F_2$  frequency-distribution into equivalent normal curves corresponding to its three modes, a fairly close approximation to a 1:2:1 ratio was obtained.

From every  $F_2$  plant an  $F_3$  family was raised, and by eye-judgment of these families the  $F_2$  plants were classified as homozygously  $P$ , homozygously  $K$ , or heterozygous, i.e.  $I$ . Repetitions of this sorting showed it to be a consistent process. Classification based solely upon measurement was the original intention but the phenomenon of "shift" despite attempts along various lines, rendered this impossible. Sorting by eye-judgment of the  $F_3$  families gave the following proportions for the  $F_2$ :  $K:I:P = 23.65:55.39:20.95$  (%). As in other investigations upon this cross which are cited, the facts suggest a one-factor difference between  $P$  and  $K$  for glume-length, the heterozygote being "intermediate" in general characters. On this hypothesis the progeny of the  $F_2.I$  (heterozygote) plants should consist of  $P$ ,  $I$ , and  $K$  plants in the proportion 1:2:1. As a fact these three types—and these only—were found and the expected ratio was again fairly closely approached. The  $F_2.K$  and  $F_2.P$  population appeared, on selfing, to breed true.

The numerical attributes of the populations into which  $F_2$  and  $F_3$  were sorted more clearly disclose the state of affairs. They are given below,



their standard errors (in all cases satisfactorily small) being omitted for economy:

$F_0 . K$ (1913) = 11.23	$F_0 . P$ (1913) = 28.60	$F_2 . I$ (1913) = 16.41
(1914) = 11.70	(1914) = 30.84	$F_3 . I$ ex $F_2 . I$ (1914) = 17.48
$F_2 . K$ (1913) = 11.42	$F_2 . P$ (1913) = 23.18	
$F_3 . K$ ex $F_2 . K$ (1914) = 11.98	$F_3 . P$ ex $F_2 . P$ (1914) = 24.66	
$F_3 . K$ ex $F_2 . I$ (1914) = 11.81	$F_3 . P$ ex $F_2 . I$ (1914) = 24.68	

It thus appears that the mean glume-length for  $P$  is in  $F_2$  "shifted" down by 24.83%, that the shifted value breeds true, and is again exhibited by  $F_3 . P$  ex  $F_2 . I$ . For  $K$  a corresponding small upward shift seems to have occurred. An examination of a number of other inheritance results which are cited, points to "shift" as a fairly common phenomenon. "Multiplying factors" have been suggested by some in explanation of the phenomena but no feature of these results appeared to lend any encouragement to that idea.

A few other facts of the earlier investigation must be noticed. Grain-length exhibited shift on lines precisely corresponding to the case of glume-length and a group of ten characters—five of the glume and five of the grain—behaved as "genetic inseparables." They appeared to be controlled as a whole by a single factor. When shift in glume-length had been established, it was decided to follow the inheritance of some other and unconnected character to see whether it displayed any abnormality. For this, solidness of straw was the best suited.  $P$  is solid (top internode),  $K$  is hollow, and in other investigations this character had presented a clear and simple mode of inheritance. Despite full observations in  $F_2$  and  $F_3$  it proved to be impossible to ascribe the experimental ratio of straw-types to any factorial arrangement. That this complexity might be associated with shift in glume-length seemed sufficient ground for continuing the study of straw inheritance into further generations.

When an  $F_1$  plant is self-fertilized one type of its zygotes is factorially identical (on the 1:2:1 basis here adopted) with those correspondingly produced by an  $F_0 . P$  plant. But the zygote formed upon the  $F_1$  is nourished by a plant factorially different from  $F_0 . P$  and of lesser general dimensions (rachis-length, etc.). A zygote, factorially  $F_0 . P$ , may thus be supposed to become imperfectly developed since the mother plant is different from  $F_0 . P$ . It may consequently grow into an inferior "shifted" plant. A population of such plants ( $F_2 . P$ ) will have a mean glume-length of value shifted below that of  $F_0 . P$ . Correspondingly for  $F_2 . K$  an upward shift may be predicted. On these lines a crude

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mechanical explanation of shift is presented. It carries many implications and these—since no other explanation can readily be suggested—seemed to warrant further examination.

### § II. *The Objects of the Investigation.*

These were directed towards the testing of the following matters:

( $\alpha$ ) The purity of type of the parental forms. Every feature of the growing and of the metrical attributes of  $F_0.K$  and  $F_0.P$  in 1912-3-4 fully supported the belief that these were, to the extent of possible determinations, pure lines. But since impurity in one or both of them might conceivably produce results simulating genuine shift, further tests seemed desirable.

( $\beta$ ) The constancy of the shifted values in successive generations produced by the selfing of  $F_2.K$ ,  $F_2.I$ ,  $F_2.P$  and their progenies. One implication of the theory indicated at the end of § I is a progressive "recovery from shift."

( $\gamma$ ) The validity of the unifactorial difference for glume-length between  $P$  and  $K$  for which the earlier investigation vouched.

( $\delta$ ) The mode of inheritance of solidness of straw and its possible connection with shift of glume-length.

( $\epsilon$ ) The investigation of grain-length was terminated with the  $F_2$  as was that of  $K$  glume-length. These two attributes appeared to behave in strict analogy to  $P$  glume-length but displayed far less proportionate shift. It was desirable to concentrate upon the clearest feature rather than to disperse observations upon four similar ones.

### § III. *Procedure.*

Up to and including the  $F_3$ , measurements of glume-length were made to 0.5 mm.: but for statistical purposes it proved necessary to limit the number of classes of the distributions by regrouping into 1.0 mm. classes. To this fact are due the fractional frequencies of Tables I—X. In  $F_4$  and  $F_5$  measurements were made to 1.0 mm. and correspondingly the frequencies of the Tables concerned (XI—XIX) are integral.

The method of sorting into types was that formerly employed. Every plant in every generation was classified as  $K$ ,  $I$ , or  $P$  according to the progeny it yielded on selfing. If the progeny plants were all, to the eye, of  $K$  (or  $P$ ) type, the progenitor plant was classified as  $K$  (or  $P$ ): if they were of all three types it was classified as  $I$  (heterozygous). Further, to

test this eye-sorting, every generation was classified immediately after harvest and the result so obtained was compared with that derived from the progenies—the next generation. Exact agreement was in all cases recorded so that the classifying was as accurate as eye-judgment could ensure. Upon the first sorting, confirmed by examination of progenies, the data were grouped for the calculation of the mean glume-lengths of the various types.

*Numbers of Tables.*

Class Means	I	II	III	IV	V	VI	VII	VIII	IX	X
7.5	—	—	0.5	1.0	—	—	—	—	—	—
8.5	3.0	—	3.0	2.5	—	—	—	—	—	—
9.5	36.0	12.5	60.5	22.0	—	—	—	—	—	—
10.5	74.0	28.5	221.0	51.0	—	—	—	—	—	—
11.5	118.5	44.0	357.0	89.0	—	—	—	—	2.0	1.0
12.5	105.0	20.0	329.5	75.0	1.0	—	—	—	2.5	4.5
13.5	51.5	6.0	184.0	36.0	—	—	—	0.5	19.0	18.5
14.5	12.0	1.0	68.5	12.0	—	—	2.5	1.5	37.5	42.5
15.5	—	2.0	13.0	2.0	—	—	9.0	1.0	44.0	69.0
16.5	—	—	—	1.0	—	2.0	9.5	3.5	73.0	94.5
17.5	—	—	—	—	—	2.0	18.5	3.0	40.5	110.0
18.5	—	—	—	—	—	1.0	31.0	6.0	34.0	106.5
19.5	—	—	—	—	—	6.5	49.0	13.5	7.0	61.0
20.5	—	—	—	—	2.0	11.5	50.0	15.5	3.5	44.5
21.5	—	—	—	—	0.5	12.0	86.5	19.5	1.5	18.5
22.5	—	—	—	—	6.0	14.0	101.0	26.0	0.5	8.5
23.5	—	—	—	—	16.0	9.0	96.5	17.5	1.0	1.0
24.5	—	—	—	—	24.0	20.0	112.5	34.5	—	—
25.5	—	—	—	—	14.5	6.0	102.0	40.0	—	—
26.5	—	—	—	—	34.5	9.0	106.0	36.5	1.0	—
27.5	—	—	—	—	38.5	5.0	78.0	33.0	—	—
28.5	—	—	—	—	39.0	1.0	72.0	19.5	—	—
29.5	—	—	—	—	48.0	1.0	53.0	18.0	—	—
30.5	—	—	—	—	66.5	1.0	28.0	13.0	—	—
31.5	—	—	—	—	63.0	—	18.0	6.5	—	—
32.5	—	—	—	—	58.0	—	17.5	2.0	—	—
33.5	—	—	—	—	69.5	—	6.0	4.5	—	—
34.5	—	—	—	—	45.0	—	3.0	3.0	—	—
35.5	—	—	—	—	33.0	—	1.0	—	—	—
36.5	—	—	—	—	20.0	—	2.0	—	—	—
37.5	—	—	—	—	11.5	—	0.5	—	—	—
38.5	—	—	—	—	6.0	—	—	—	—	—
39.5	—	—	—	—	2.0	—	—	—	—	—
40.5	—	—	—	—	1.0	—	—	—	—	—
Totals	400	114	1237	292	600	101	1054	318	267	580

A separate investigation [Engledow and Shelton (3)] had emphasised the importance of restricting observation upon cereal plants to populations having a constant (the modal) number of tillers. In consequence this modification was introduced in the 1921 measuring (for  $F_5$ ). It is fully explained in connection with that generation.

The  $F_2$  and  $F_3$  populations were complete: that is to say every  $F_1$  and in turn every  $F_2$  plant was grown on. But a complete  $F_4$ —still more a complete  $F_5$ —would have been so large that it could not have been

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measured for glume-length in the time available. Consequently a partial  $F_4$  and a partial  $F_5$  were grown, a course which naturally involved the selection of certain  $F_3$  and  $F_4$  plants to the exclusion of the remainder. Such a selection, inevitable unless resources of time and assistance be very considerable, must, and in all similar work inevitably does, invite

*Numbers of Tables.*

Class Means	XI	XII	XIII	XIV	XV	XVI	XVII	1920 XVIII	1921 XVIII	XIX
10	—	—	—	—	—	—	—	—	—	2
11	—	—	—	—	—	—	—	—	—	6
12	2	—	1	—	—	—	—	—	—	15
13	1	—	1	—	2	1	—	—	—	16
14	3	4	—	1	—	1	1	—	—	36
15	3	2	2	6	8	10	3	1	—	35
16	1	4	7	6	7	10	7	1	—	35
17	6	20	13	7	10	11	3	—	—	34
18	3	21	9	4	16	9	9	4	—	22
19	3	30	31	7	22	13	10	10	1	6
20	7	37	32	12	19	25	13	15	10	3
21	12	21	42	14	23	15	28	16	7	2
22	12	48	63	12	25	21	26	35	7	—
23	15	53	76	8	34	21	22	46	11	1
24	16	46	78	11	15	24	20	61	10	—
25	6	58	83	10	12	15	17	64	11	—
26	10	43	67	4	6	10	10	72	11	—
27	4	37	51	4	9	7	9	64	26	—
28	6	25	55	1	3	5	12	109	21	—
29	5	20	21	4	5	2	3	91	17	—
30	2	24	21	1	—	—	1	181	14	—
31	1	6	12	—	1	—	—	104	14	—
32	—	9	3	—	1	—	—	110	6	—
33	1	2	3	—	1	—	—	79	2	—
34	—	—	—	—	—	—	—	71	1	—
35	—	—	—	—	—	—	—	53	1	—
36	—	—	—	—	—	—	—	30	—	—
37	—	—	—	—	—	—	—	20	1	—
38	—	—	—	—	—	—	—	10	—	—
39	—	—	—	—	—	—	—	4	—	—
40	—	—	—	—	—	—	—	4	—	—
41	—	—	—	—	—	—	—	—	—	—
42	—	—	—	—	—	—	—	1	—	—
Totals	119	510	671	112	219	200	194	1206	171	213

*Tables of Frequency-Distribution of Glume-Lengths.*

Table Number	Types and Generation	Table Number	Types and Generation
I	$F_0$ . K 1914	XI	$F_4$ . P ex $F_3$ . I
II	$F_2$ . K	XII	$F_4$ . P ex $F_3$ . P ex $F_2$ . I
III	$F_3$ . K ex $F_2$ . K	XIII	$F_4$ . P ex $F_3$ . P ex $F_2$ . P
IV	$F_3$ . K ex $F_2$ . I	XIV	$F_5$ . P ex $F_4$ . I
V	$F_0$ . P 1914	XV	$F_5$ . P ex $F_4$ . P ex $F_3$ . I
VI	$F_2$ . P	XVI	$F_5$ . P ex $F_4$ . P ex $F_3$ . P ex $F_2$ . I
VII	$F_3$ . P ex $F_2$ . P	XVII	$F_5$ . P ex $F_4$ . P ex $F_3$ . P ex $F_2$ . P
VIII	$F_3$ . P ex $F_2$ . I	XVIII	$F_0$ . P 1920 and 1921
IX	$F_2$ . I	XIX	$F_5$ . I ex $F_4$ . I
X	$F_3$ . I ex $F_2$ . I		

N.B. "ex" implies derived by self-fertilization from the plant specified.

criticism. The best that can be done in the circumstances is to furnish a detailed account of the method of selection followed. Random selection is, of course, essential.

In 1919 the  $F_3$  and parental populations were:

$$\begin{aligned} F_0.P &= 600 \text{ plants} \\ F_3.P \text{ ex } F_2.P &= 1054 \quad ,, \\ F_3.P \text{ ex } F_2.I &= 318 \quad ,, \\ F_3.I \text{ ex } F_2.I &= 580 \quad ,, \end{aligned}$$

The  $F_0.P$  plants had been serially numbered as they were measured and numbers 1-150 were used to supply seed for the raising of the 1920 crop. Sampling was thus purely random. In the 150 families so raised there were 1205 undamaged plants, all of which were measured for glume-length. One family in every three was selected (Nos. 3, 6, 9, ...), making 50 families in all, and the first plant (*i.e.* the one which chanced to be No. 1) in every family furnished seed for the 1921 crop. This crop was sorted into groups according to the number of tillers per plant. The modal number was two and, in conformity with the system elsewhere explained, the plants of the modal class, 171 in number, alone were measured. A modal sample of this kind affords a measurement of mean glume-length of low probable error despite the relatively small number of observations.

• The population written as  $F_3.P \text{ ex } F_2.P$  consisted of the descendants of  $F_2$  plants which had been classed by eye as " $P$ " and subsequently confirmed in this class by examination of their  $F_3$  progenies. They were 1054 in number and comprised 101 families. Families 5, 10, 15, ... 100, *i.e.* 20 families, were taken and plants Nos. 1, 2, and 3 from every family were selected. From the 60 plants so chosen an  $F_4(F_4.P \text{ ex } F_3.P \text{ ex } F_2.P)$  of 671 plants was raised, all of which were measured. A repetition of the random selection process furnished a corresponding  $F_5(F_5.P \text{ ex } F_4.P \text{ ex } F_3.P \text{ ex } F_2.P)$  the frequency of whose modal class was 194, the plants of this class alone being measured.

For the remaining populations precisely similar methods were followed. The eye-sorting into  $K$ ,  $I$  and  $P$  types in every generation was confirmed by the examination of the families yielded by the single plants of that generation, in the subsequent one. From the "Key to Populations" (Diagram I) attached to the Tables may be seen exactly what populations were grown, the modes of their descent, the numbers of plants actually measured, their mean glume-lengths and the limits of their glume-length distributions. Full distributions are contained in Tables I—XIX.

§ IV. *Tests of Purity of the Parental Forms and of one of the Extracts.*

No genetic investigation can be valid unless the homozygosity and singleness of type of the parent forms have been demonstrated. This is particularly the case when a quantitative phenomenon such as "shift" is in question. In some instances where the experimental character is a metrical one a definitely unimodal frequency distribution for a parent form is a sufficient guarantee of purity. But the work of East (4) has proved the existence of numerous multiple factors governing a single character, and still more numerous and obscure "minor multiplying factors" are suggested by Belling's (5) results. In a population consisting of two or more close genotypes, the frequency distribution for a metrical character might well be unimodal owing to the overlap of the two components. Actually, in the earlier investigation [Engledow (1), p. 121 ( $\gamma$ )] the distribution of the complete  $F_2$  for grain-length was unimodal (it was resolved into three components by the raising of a complete  $F_3$ ). These considerations make it essential to conduct special tests of purity.

A cereal stock may be impure from various causes. The original progenitor seed may have been heterozygous, seed of a different pure line may have become admixed, and out-pollination may have occurred. Every circumstance of the successive growing-on of the parental stocks is opposed to a supposition of impurity from any one of these causes so that it remains to make a definite metrical test. Conceivably a great number of multiple factors, producing individually a very small result, may be involved. Their very existence could readily be masked by fluctuations, and although it is safer to make a reservation concerning this possibility, nothing further can be done with them. Fluctuation and the limitations of statistical method make it possible to test only for an order of impurity which may be great in comparison with the imaginable effects of minor factors.

The parental  $P$  stock was tested for purity in the following way. As explained in § III above, 150 plants (1 ear per plant) were selected at random and from these 150 families were obtained in 1920. If the parental stock were pure, then the standard deviation displayed by the family-mean glume-lengths of the 150 families should be practically the same as that calculated for the mean of a sample of one family. For the calculation the formula would naturally be  $\sigma/\sqrt{n}$  where  $\sigma$  = standard deviation (for single plants) of the complete population constituted by the 150 families and  $n$  = size of a family. Clearly " $n$ " will not be con-

stant so that the harmonic mean of its values may be used. The number of plants per family has a range of 1 to 19. This is too wide to permit any number (harmonic mean or other) being fairly adopted as the "number of plants per family" in the evaluation of  $\sigma/\sqrt{n}$ . Consequently the families lying between 6 plants and 12 plants per family were taken. They were 80 in number and thus distributed:

Number of plants per family	= 6	7	8	9	10	11	12
Number of families	= 13	7	15	12	10	13	10

The standard deviation of the means of these 80 families, calculated in the usual way, was 1.77 mm. For the single plants of the complete parental population the standard deviation was 4.316 mm. The harmonic mean (weighted) of the number of plants per family (for the 80 families lying between 6 plants and 12 plants) being denoted by  $H$ , calculation gives:

$$\frac{1}{H} = 0.11738.$$

The standard deviation of the mean of a family of  $H$  plants, being denoted by  $\sigma$ , is:

$$\sigma^2 = \frac{(4.316)^2}{H} = 2.1865,$$

$$\therefore \sigma = 1.48 \text{ mm.}$$

This is the value to be expected on the assumption that the parental  $P$  stock is of one type. As shown above, the actual value is  $\sigma = 1.77$  mm. Taking into account the sizes of the families and the other circumstances, the agreement appears to be sufficiently good to justify the conclusion that, in the light of this method of test, the parental  $P$  stock is of one type for glume-length.

The following simple test may also be imposed. Between the means of groups of families, the greatest difference is:

13 families each of 6 plants (= 78 plants) have jointly  $M = 29.91$ .

13 " " " 11 " (= 143 " ) " "  $M = 28.47$ .

Difference = 1.54 mm.

If the population be of one type only and the error of the difference of two such means be denoted by  $\epsilon$ , then:

$$\epsilon^2 = (4.316)^2 \left( \frac{1}{78} + \frac{1}{143} \right),$$

$$\therefore \epsilon = 1.586.$$

The greatest inter-group mean difference is thus less than the corresponding standard error of sampling. Purity of the parental stock is therefore confirmed by this subsidiary test.

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Corresponding tests of parental *K* stock were made. The families selected lay between 8 and 16 plants per family, and were 100 in number. Their mean glume-lengths gave a standard deviation of 0.632 mm., the expected value for a family of "*H*" plants being 0.371 mm. The difference is considerable and demands examination.

Let  $\sigma_e$  = value expected for a family of "*H*" plants on the assumption of uniformity of type.

$\sigma_f$  = value found for the 100 families.

$$\sigma_r^2 = \sigma_f^2 - \sigma_e^2.$$

Now  $\sigma_e^2$  = variance to be expected from the errors of sampling so that  $\sigma_r^2$ , the residual variance, is the amount which has to be ascribed to special causes. Soil-differences constitute, in this case, a special cause: for all the plants of a family are grown in one drill (*i.e.* in general, on one "patch" of soil). On that account it is to be expected that  $\sigma_f$  will exceed  $\sigma_e$ . The value of  $\sigma_r = 0.512$ , *i.e.* about 4.5% of the mean glume-length. This value, examined in the light of, for example, inter-seasonal mean-differences, is not of great significance. Thus for parental *K* stock in 1914 the mean of 400 observations was  $11.70 \pm 0.04$ , while in 1920 the mean of 1751 observations was  $11.38 \pm 0.03$ —a difference of about 2.8% of the mean.

In cases of this kind there is no obvious and precise criterion. It will be assumed that, having regard to the whole of the evidence, the parental *K* stock may be accepted as of one type. Admittedly the value of  $\sigma_r$  arouses misgivings, but it appears to be a matter of great difficulty to convert them into positive statements upon the impurity of the  $F_0$ . *K*: still more is it difficult to carry them forward into an exact criticism of the phenomenon of shift as described in the earlier investigation. To give the complete evidence has seemed the only course.

At the time of the earlier investigation the purity of type of  $F_2$ .*P* was tested on lines different from those above described, but the results were not then published. It is clear that if  $F_2$ .*P* be of one genotype the differences observed among its individual plants are fluctuations, and will not be systematically transmitted when  $F_2$ .*P* is produced by self-fertilization. A test may be made by evaluating the coefficient of correlation between the  $F_2$ .*P* plants and their  $F_3$ .*P* progenies. Two forms of correlation suggest themselves, *viz.*: (i) between the  $F_2$  glume-length and the mean glume-length of the  $F_3$  family, and (ii) between the  $F_2$  glume-length and the glume-lengths of the separate plants of the  $F_3$  family, the  $F_2$  value being entered in the correlation-table once for every plant of the  $F_3$  family. Calculation shows that the value of the



coefficient of correlation for method (i) =  $0.22 \pm 0.09$ . This value is low, and in terms of its own probable error not fully significant. Since the value of the correlation given by method (ii) will be still lower, it is concluded that, by the method employed, the singleness of type of  $F_2$ .  $P$  has been demonstrated.

§ V. *The Inter-parental Factorial Difference in regard to Glume-Length and the Permanency of the "Shifted" Values.*

For determining inter-parental difference the familiar procedure is to classify the  $F_2$  plants, obtain the ratio of the types, and then test the  $F_2$  classification by raising from every  $F_2$  plant an  $F_3$  family. Though ordinarily satisfactory, this is inadequate here. The  $F_2$  comprises three types, two with strong general resemblances to the respective parents and one apparently an intermediate: the ratio is approximately 1:2:1. But in regard to mean glume-length the seemingly parental types differ quite markedly from the  $F_0$ , i.e. they display "shift." Multiple-factors or some other complication may consequently be suspected. It is necessary, then, to carry the populations beyond the usual  $F_2$  stage testing, in every generation, the ratio of types produced by the selfing of the "intermediate" class (heterozygous and denoted by  $I$ ) and the fixity of type of all the extracts. The two required tests are intimately associated so long as the possibility of numerous multiple factors is admitted. But since the evidence of the earlier investigation supported the simple hypothesis of a one-factor glume-length difference coupled with a permanent (though inexplicable) shift, it seems better first to test this explanation. Subsequent search can be made (§ VII below) for multiple factors. It is not inconceivable that numerous "minor multiplying factors" are operative in such a manner as to produce, by the overlap of distributions, a good simulation of unifactorial effect.

First, then, must be considered, the actual ratios of the  $K$ ,  $I$  and  $P$  types in the progenies of populations classed as  $I$  (and therefore regarded as heterozygous). These were as shown below, the first line giving the percentage of the type, the second the observed deviation from the simple expectation [ $25\% : 50\% : 25\%$ ], and the third line the standard error attributable to sampling.

Unfortunately the record of the progeny of  $F_3$ .  $I$  has been lost, and since, as explained in § III (above), the complete progeny was not grown on, it was impossible to deduce the facts from the  $F_4$  records. Actually the ratio in question was a closer approximation to 1:2:1 than any of those given above. In the absence of the facts this evidence, furnished

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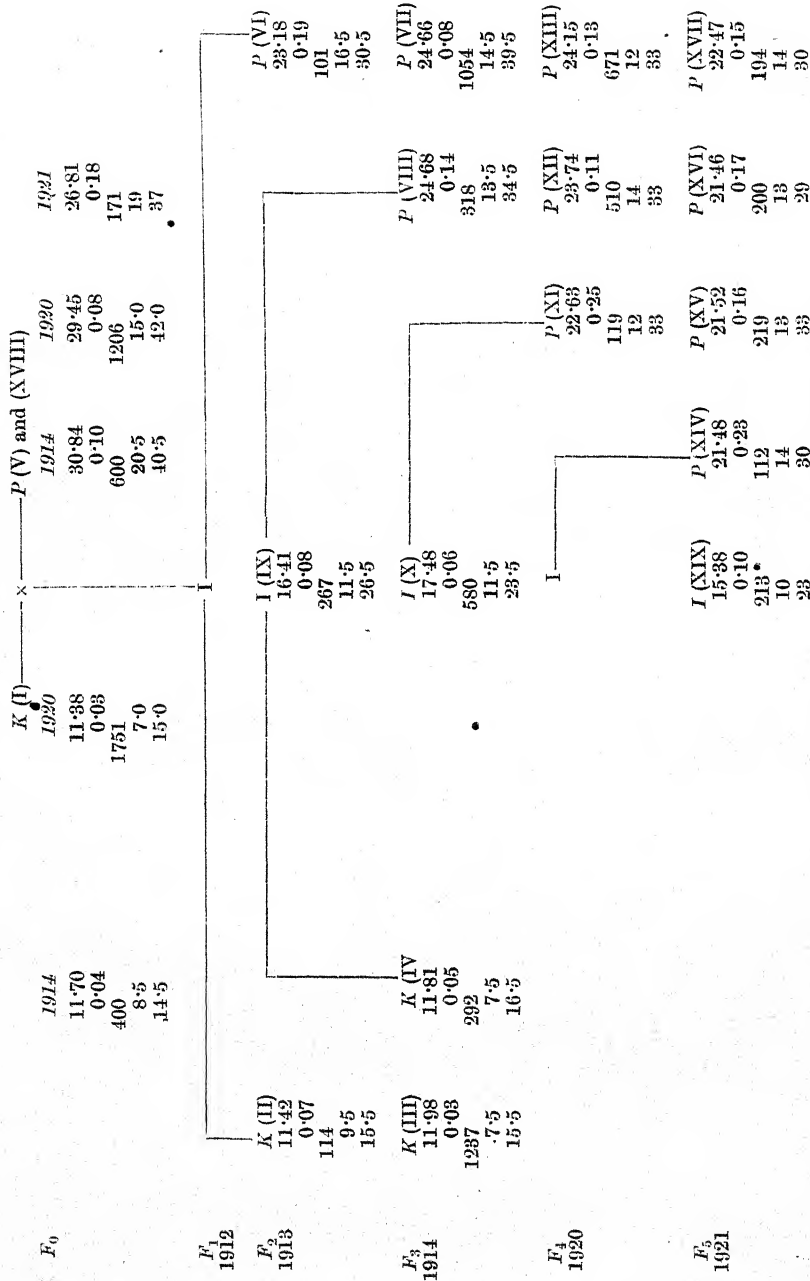
simply from memory, can do no more than to suggest that the data actually given deserves its full face-value. Bearing in mind that the errors of sampling (third line of each row of the table) are *standard errors*, it may be observed that of the nine differences from expectation involved 5 are less than the relevant error, 2 lie between 1.0 and 2.0

Progeny of	Types			No. of plants in Progeny
	<i>K</i>	<i>I</i>	<i>P</i>	
$F_1 \cdot I$ }	23.65 1.35 1.97	55.39 5.39 2.28	20.95 4.05 1.97	482
$F_2 \cdot I$ }	24.54 0.46 1.25	48.74 1.26 1.45	26.72 1.72 1.25	1190
$F_4 \cdot I$ }	26.32 1.32 1.16	49.09 0.91 1.34	24.58 0.42 1.16	1383

times the error and 2 between 2.0 and 3.0 times the error. None therefore reaches 3.0 times the error. The most suspicious deviations are exhibited by the progeny of  $F_1 \cdot I$ . Thus on the whole there is statistically satisfactory agreement with the 1 : 2 : 1 expectation which implies a unifactorial difference between the parents for glume-length. It seems proper to observe that statistical guarantees are not, with absolute invariability, biologically acceptable. A published instance [Engledow (6)] illustrates this. This reservation applies in principle, however, to the generality of Mendelian results, and has no special significance in the present instance.

In some other investigations upon glume-length inheritance, the strain of  $F_0 \cdot P$  here employed was a parent. The results deserve notice in so far as they bear upon the validity of the 1 : 2 : 1 ratio. Biffen (7) made reciprocal Polish  $\times$  Rivet crosses. The  $F_2$  glume-length distribution fell into three groups so sharply separated that the frequencies could be determined without further analysis. In percentages they were: Rivet-type : Intermediate : Polish Type = 25.04 : 51.09 : 23.87 in one direction and 24.48 : 52.36 : 22.78 for the reciprocal. Backhouse (8), for the  $F_2$  of the Polish  $\times$  Kubanka cross, experienced difficulty in separating the constituents of the glume-length distribution. He derived the ratio  $K : I + P = 1 : 3.13$ . Caporn (9), in the case of Polish  $\times$  Eloboni, concluded that three types segregated in  $F_2$  and that the ratio was 1 : 2 : 1. His  $F_2$  was of 183 plants only. These results naturally do not directly support the conclusions for the Polish  $\times$  Kubanka cross which is under consideration. That they are in harmony with those conclusions is nevertheless important.

DIAGRAM I. Key to Populations.



The values for every population are in vertical order: Mean glume-lengths—probable error of mean—number of observations—lower limit of distribution—upper limit.

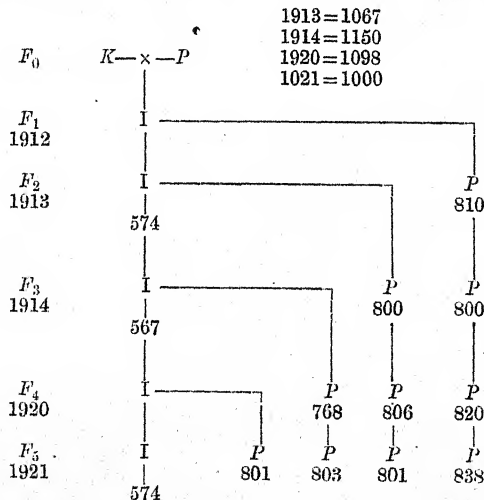
\* The Roman Numerals indicate the corresponding tables of frequency-distribution of glume-length.

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Consideration must now be given to the mean values of glucose-length in the various  $P$ -extracts of the successive generations. Measurements of  $K$  were not carried on beyond  $F_3$  for reasons which have been given, so that extracts of this type receive no further notice. A general inspection shows that in all cases (see Diagram I) the probable error of the mean is relatively small: it follows that the number of observations was adequate throughout. Wide differences in the upper and lower limits of range are displayed, but these do not signify. The larger the sample, the wider does the range of distribution in general become. A striking feature is the seasonal fluctuation of  $F_0.P$ . This is evident in Diagram II, where the annual mean values are expressed in terms of the lowest (1921) as 1000. Presumably the very low value reflects the effects of the prolonged drought of that year. The extremes of inter-season difference are roughly 3% and 15% of the mean. It becomes necessary, for comparative examination, to express the mean value of every extract in terms of the mean of  $F_0.P$  for the year concerned taken as 1000. This is done in Diagram II which, in form, corresponds to Diagram I: its mean values may be called "corrected means."

DIAGRAM II.

*Key to Corrected Means.*



For  $F_0 \cdot P$  the annual values of mean glume-length are given in terms of the 1921 value as 1000.

For all the extracted populations the mean is given in terms of the mean of  $F_0$ .  $P$  for the year concerned as 1000.

The *I* extract (intermediate and, from the conclusions of § IV, heterozygous) remains singularly constant from generation to generation, the difference in corrected means being only 1.2%. From this it is inferred first that the unifactorial difference of the parents is substantiated: and further, that a stable condition is reached in  $F_2$  and persists at any rate to  $F_5$ . There is no repetition of "shift" at *I*-plant zygosis after  $F_1$ . *I*. Measurements of  $F_4$ . *I* (1920) were not made because of the time required for the 7000 measurements made in that year on other extracts.

Broadly speaking, the whole of the *P* extracts ( $F_2$ — $F_5$ ) of Diagram II centre round the value 800. As a group therefore they display a "shift" from  $F_0$ . *P* of 200, i.e. 20% of  $F_0$ .  $M_P$  which, whatever complications may later appear, is a highly significant effect. For a single generation the greatest difference between the means of two extracts is that shown in  $F_4$ , concerning which the facts are:

Extracts	$M(F_0, P=1000)$	$M$ actual	$\sigma$	Number of Observations
$F_4$ . <i>P</i> ex $F_3$ . <i>I</i> ...	768	22.63	4.158	119
$F_4$ . <i>P</i> ex $F_3$ . <i>P</i> ex $F_2$ . <i>P</i>	820	24.15	3.410	671

Assuming that the two extracts are of the same genotype, the standard error of the difference of their means (using  $\sigma$  and number of observations given above) is 0.403. The actual difference is  $24.15 - 22.63 = 1.52$ , which exceeds three times the standard error. It will be seen, however, that even if the extracts be of the same genotype, the test imposed on the difference of means is very drastic. It makes no allowance for the fact that the extracts were grown on different areas of ground. For the test to be a fair one, the two extract-populations should have been mingled or in some manner fairly dispersed over the same area. The other considerable inter-extract difference ( $838 \sim 801$  in  $F_3$ ) is within satisfactory range of the appropriate error evaluated as in the former case. Inter-seasonal fluctuation is shown by the  $F_0$ . *P* means to be very marked and therefore, in principle, considerable inter-soil-area effects in one year may be expected. If this be accepted as a counter-balance to the discrepancy (deviation from error attributable to sampling) which has been pointed out, the conclusion must be that all the *P*-type extracts of Diagram II are of one genotype. Coupled with the validity of the 1 : 2 : 1 ratio, this is a confirmation from the observation of  $F_4$  and  $F_5$ , of the deductions from the earlier investigation: "Either *P* or *K* possesses a factor for glume-length which the other lacks and the heterozygote is, broadly speaking, intermediate between the parental forms... the parental *P* does not re-appear in  $F_2$ . In its place are found *P* plants

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which closely resemble  $F_0.P$  in general appearance, but whose mean glume-length is more than 20% lower than that of  $F_0.P$ . This "shifted" form, when selfed, breeds true." It will be recalled that the purity-test of  $F_2.P$  described in § IV (above) is an additional confirmation.

The implications of the crude explanation of "shift" suggested in § I (*supra*) are in harmony with the trend of the values of Diagram II. But they relate to the behaviour of shifted values in the repeated propagation of homozygous extracts, and are therefore relegated to a later paragraph.

### § VI. *Straw and Endosperm in the Cross.*

In the earlier investigation it appeared to be desirable to study the mode of inheritance of the straw. One parent ( $P$ ) has solid straw, the other hollow. Three types were found in  $F_2$ , the analogues of the  $F_0$  forms and a semi-solid or intermediate. By inspection of the  $F_3$  families it was possible to grade the  $F_2$  plants as  $H$  (homozygously hollow like  $F_0.K$ ),  $S$  (homozygously solid like  $F_0.P$ ), and  $HS$  (semi-solid or intermediate and, as the progeny showed, heterozygous). For the complete  $F_2$  so graded, the ratio  $(H + HS) : S$  was sharply 3 : 1 but the ratio  $H : HS : S$  had varying values for  $F_2.K$ ,  $F_2.I$ ,  $F_2.P$  and  $F_2(K + I + P)$  none of which was a good approach to 1 : 2 : 1. In addition to this peculiarity was the fact that of a group of other results on glume-length inheritance, one alone gave no evidence of "shift" [Biffen (7)] and this was the only one in which both parents had solid straw, the other crosses having been Hollow and Solid [Backhouse (8), Caporn (9)]. The intrinsic complexity and the speculative possibility of its association with shift, prompted further prosecution of the question of straw-inheritance. Additional data afforded by  $F_4$  and  $F_5$  failed to cast new light upon the  $H : HS : S$  ratio. It fluctuated considerably as in  $F_2$  and  $F_3$ . Moreover, the  $(H + HS) : S$  ratio in the populations derived from  $F_2.I$ ,  $F_3.I$ , and  $F_4.I$  was less definitely in agreement with 3 : 1 than in the  $F_2$ . A complicated mode of inheritance seemed still possible: but it had also become apparent that the differentiation of the  $H$  and  $HS$  types was often arbitrary. This led to investigation of the more precise nature of "solidness" and "hollowness" of straw. The customary method—examination of the top internode at a few inches below the ear—proved unreliable in the case of poorly developed plants. It was necessary to examine every internode. Observation on this basis was therefore made on the  $F_4$  but again consistent results were not obtained. To gather further experience of straw-characters two other suitable crosses, which happened

to be available, were studied. One was Polish  $\times$  Rivet in which both parents are solid in the "neck" (i.e. just below the ear) but Polish remains fully solid almost to the base of the first internode at which point Rivet is only "half-solid." In the  $F_2$  four degrees of solidness appeared—the two parental, more solid than either parent, and less solid than either parent. Grouping these degrees singly as  $H$  and  $S$  the clear-cut 3:1 ratio established by Biffen (7) was obtained. The second cross was Rivet  $\times$  Chinese White (i.e. *T. turgidum*  $\times$  *T. vulgare*) of which the second parent has, relatively speaking, clearly hollow straw. A type more solid than Rivet was found in  $F_2$  but for the simple  $H:S$  ratio a value close to 3:1 was found. It is to be expected that solidness of straw is not morphologically a simple character. Not only the presence of a greater or less amount of "pith" but also the girth and other characteristics of the surrounding wall of the straw, must determine the relative solidness. This was clearly seen to be the case on close inspection of the plants of the  $F_2$ 's in question. Thus the unexpected types found in the  $F_2$ 's of these crosses and the inconstancy of the ratios exhibited by the several generations of the Polish  $\times$  Kubanka cross, seem attributable to the complex nature of "solidness of straw." Finally, for the extracted populations of  $F_4$  and  $F_5$ , e.g.  $F_5.P \text{ ex } F_4.P \text{ ex } F_2.P \text{ ex } F_2.I$ , the mean glume-lengths for the  $S$ ,  $H$ , and  $HS$  sub-types were determined. In no case was a significant difference found. It is concluded therefore that straw-inheritance in the Polish  $\times$  Kubanka cross possesses no feature of special interest and none which bears upon "shift" in mean glume-length.

The endosperm in inheritance was a side-issue of the earlier investigation. Jensen (2) having now demonstrated double-fertilization in wheat, it is of great interest to try to harmonise observations upon hybrid endosperms with the corollaries of this observation. The first corollary is that the grains borne by an  $F_1$  plant (and similarly by an  $F_2.I$ , etc. plant) are a generation ahead of the plant itself. Thus, among them, three types of endosperm should occur for the  $P \times K$  cross corresponding to the three types of embryo. If formative significance attaches to the "double-dose of femaleness" in the fertilized endosperm-nucleus, then, as a more refined corollary, four endosperm-types should exist. Measurement of the grains borne by  $F_2.I$  plants gave evidence of one type only. Overlap of distributions might obliterate the existence of four component types if these had close mean-values. Consequently extensive measurements of length were made upon the grains borne by  $F_3.I$  plants, but again there appeared to be but one

length-type. Attempts on various lines were made to analyse the complete  $F_3$ .  $I$  grain population into types, *e.g.* growing-on grains of observed length to find out the genetic constitution of their embryos so that mean grain-lengths corresponding to the three embryo types might be found. Negative evidence only was obtained, and it was clear that no really significant evidence of any kind could be secured save by a very big series of observations—as big perhaps as the whole of the rest of the investigation. In the circumstances the maternal nature of the endosperm is still presumed but no suggestion can be proffered for the harmonisation of this view with triple-fusion.

This interesting feature of the wheat-endosperm was pointed out by Biffen in 1905. Since then it has appeared in many wheat crosses and affects a wide range of characters. Two series of crosses alone bear any trace of being exceptional. In *T. turgidum*  $\times$  *T. vulgare* crosses the  $F_1$  plant bears perfect, wrinkled, and completely shrivelled (non-germinable) grains. Further study is necessary, however, before this can be accepted as a divergence from singleness of type in the special sense we are considering. Again, the  $F_1$  plants of *T. turgidum*  $\times$  *T. durum* may bear grains which are "vitreous" (like *T. durum*), all "starchy" (like *T. turgidum*), or "mottled" (starchy and vitreous in patches). But here, too, caution is necessary: for occasional grains of *T. durum* may be mottled or even starchy. In inheritance, the endosperm of wheat remains an interesting enigma.

### § VII. *The Nature of "Shift."*

It is by no means uncommon to find the frequency distribution of a character in the  $F_2$  of a cross, passing beyond the extreme limits of the two parents combined. Or, by contrast, in other cases the limits of  $F_2$  range may not reach even the mean values of the parents. In both these sets of circumstances it is generally accepted that the character concerned is governed by several factors, called then "multiple factors." To the extent to which hypothetical Mendelian factors in general can be reasonably imagined, it is not very difficult in many of these cases to conceive the possibility of 2, 3, or even 4 factors all controlling a single plant "character." To varying degrees in a number of crosses, the breeding of partial  $F_3$ ,  $F_4$ ... generations has supported the multiple-factor hypothesis. This hypothesis evades stringent tests because of its elasticity. The number of factors and their individual potencies can be endlessly varied, and thus made to meet a great diversity of observational requirements. But the more numerous the stipulated factors, the more



numerous must be the observations required for the proof of their existence. It is perhaps not unduly cautious to say that investigations relating to multiple-factor inheritance have so far indicated rather than demonstrated the existence of such factors. In regard to some investigations the indications are exceedingly strong and the multiple factor hypothesis must be accepted because it is by far the simplest in the particular circumstances. But a possible alternative appears to exist. Though its application may be limited it is, if substantiated, of great importance. Castle's much debated work on rats prompted the idea of the "modification of unit characters by selection": and Gates (10) in 1915 was led to insist that in the events following crossing, gametic purity was not always to be expected. The non-committal word "shift" was adopted in an earlier publication [Engledow (1)] to meet the requirements of the Polish  $\times$  Kubanka cross. In this cross it was found impossible to devise a suitable multiple-factor explanation. Other cases were cited (*loc. cit.* p. 118) in which "shift" seemed equally to have application. A few more might be given and particularly some of qualitative characters in wheat. Thus Biffen in the Polish  $\times$  Rivet cross found that the characteristic "grey" of the Rivet chaff never again appeared even up to  $F_6$ . The use of the term shift in no way militates against the idea of multiple-factors—it is no more than a reservation to secure further attention to certain crosses in which a separate phenomenon may possibly be displayed.

The apparent instances of "shift" already mentioned, seem at first sight to demand a multiple-factor explanation. For the thorough testing of such an explanation it would be necessary to raise very great numbers of separate families in  $F_3$ ,  $F_4$ ,  $F_5$ ... so that the gradual separation of distinct homozygous strains could be demonstrated. If even six factors were involved the work would be almost endless: and further, if the factors each produced a small effect, inevitable fluctuation might make it impracticably difficult to separate two types differing by only one factor. In the circumstances, therefore, the best procedure is perhaps to test the "shift" explanation upon an accepted multiple-factor case and conversely. The *Nicotiana* crosses of East (4) are one of the best examples of careful multiple-factor explanations. If it be assumed that the parents differ by but one factor, it must be said that his  $F_3$  family 1—1 corresponds to the  $F_2$ . *I* of Polish  $\times$  Kubanka. Families 1—2, 1—3, 1—4, and 2—5 correspond to  $F_2$ . *K* and display upward shift: while 2—1, 2—3, 2—4, and 2—6 correspond to  $F_2$ . *P* and display downward shift. His  $F_4$  families are only four in number, his  $F_5$  but two.

More would be desirable, but at any rate these show a distinct rise in mean values above the  $F_2$  families which he grew. If shift be permanent (*vide* § V *supra*) then it has no application to East's results. As is briefly indicated below a recovery from shift may be predicted from a very crude theory as to the nature of shift but insistence upon this point is not warranted. East's work falls short of a thorough vindication of the existence of multiple factors but it points to such an explanation rather than to shift. In the absence of more data nothing further can be said. The multiple-factor explanation has now to be applied to a case reserved under the name of "shift" for which Polish  $\times$  Kubanka will serve.

A distinctive method was followed in this investigation. The  $F_2$  plants were classified by eye as  $K$ ,  $I$  and  $P$  (confirmed by  $F_3$  progenies) and for this reason direct comparison with results such as those of East is difficult. It is necessary to consider the salient features of the results which proceeded from this method—a 1:2:1 ratio in the progenies of successive  $I$  populations, and a constant value of  $M_I$  (*vide* § V *supra*). It may be supposed that these features represent the combined working of a principal factor ( $A$ ) and a minor factor ( $B$ ). The eye, unable to appreciate small differences, might sort the main  $A$  types in a population bred from  $I$  plants and simply disregard the subtypes representing varying superposed  $B$  effects. Such an assumption accords with the distinct trimodality of the  $F_2$ , and of the complete progeny of  $F_2.I$ . For example, if the parents were  $F_0.K = aabb$   $F_0.P = AA BB$  then the eye-determined  $F_2.P$  population would be  $AA (BB + 2Bb + bb)$ . Shift in  $F_2.P$  and  $F_2.K$  would be expected in the directions actually observed. But there is an obvious difficulty, for the  $F_0.P$  type should constitute 25% of  $F_2.P$ , and clearly this was not the case. By postulating more factors  $C$ ,  $D$ , ..., and perhaps ascribing certain selected glume-length potencies to them, the possibilities of explanation are readily enlarged. Suitable postulations might account for the constancy of  $M_I$ , but there remains the range of variation of the successive types, *e.g.*,  $F_3.P$  ex  $F_4.P$  ex  $F_3.P$  ex  $F_2.I$ , etc. (*vide* Diagrams I and II). With several factors  $B$ ,  $C$ ,  $D$  ... the limits of total range,  $\sigma$ , and  $V (= \sigma/M)$  would progressively alter. The form of the progression may, of course, be calculated for specified factorial arrangements. Actually there is no evidence of progression. Such differences in value as occur are irregularly distributed. It therefore appears necessary to seek a more elaborate form of multiple-factor explanation for these results. Several different theories were attempted. By assuming the occurrence of principal and minor

factors (*vide supra*), of "additive" and "multiplicative" factors and so on, numerous possibilities were opened. Since none of these, however, could be brought into harmony with the observed facts, it is needless to discuss them. It is concluded that there is still considerable presumptive evidence of the existence of a phenomenon not attributable entirely to multiple-factor effect and whose form is illustrated by the instances to which the term "shift" has been applied.

The developments of the chromosome theory suggest possibilities which might apply to otherwise unaccountable cases of shift. At present, however, the chromosome numbers of  $F_0.P$  and  $F_0.K$  have not been determined. As indicated in § I an explanation of shift may be based upon the assumption that the development of a zygote is dependent upon the genetic constitution of the mother plant. Thus  $F_2.P$  plants, developing from zygotes formed upon  $F_1.I$  plants, will be impoverished in comparison with genetically identical zygotes borne by  $F_0.P$  plants. The impoverishment might find expression in a downward shift of the mean glume-length of the  $F_2.P$  population. In such an idea there are several implications. For example, the  $F_1$  plants from reciprocal  $P \times K$  crosses might be expected to differ in mean glume-length. In connection with the mean values of Diagram II it may be noted that a "recovery from shift" is to be predicted. The  $F_2.P$  plants are genetically identical with  $F_0.P$  and though of a smaller mean glume-length, may be expected to bear better-nourished zygotes than those of  $P$  type which are produced on  $F_1.I$  plants. Consequently the mean value for  $F_3.P$  ex  $F_2.P$  should exceed that of  $F_2.P$ . In other lines of descent similar evidence of "recovery" is to be anticipated from this argument. The sequence of the right-hand column of Diagram II is at first sight suggestive in this connection, but, as the discussion of § V (*supra*) makes clear, the differences constituting the sequence may be attributable to errors of sampling.

The existence of shift as a phenomenon distinct from multiple-factor action remains, after the experimental work here recorded, a matter of uncertainty. Apart from the bare fact of occurrence, the same may perhaps be said of multiple-factors even in such careful investigations as those of East. The data presented in Diagram I represent over 10,500 measurements of glume-length. Many subsidiary measurements had to be made and these, together with grain-length observations, make a total of nearly 30,000 measurements. These numbers prompt a speculation upon the probable experimental requirements of an endeavour to repeat the investigation on such a scale as would be likely to settle the

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true nature of shift. Breeding would probably have to be continued to  $F_3$ . If several multiple factors be responsible for the effect and if these have all about the same influence on glume-length families (single plant progenies) would have to be kept separate in every generation. Now if the average strength of a family be ten plants, a single  $F_2$  plant would yield 10,000  $F_3$  families. It would be useless to grow and measure one such set of families, e.g. from an  $F_2$   $P$  plant. For if constancy were obtained throughout, the fact might represent shift of a permanent nature, or it might simply imply that multiple-factors were present, the selected  $F_2$  plant being by chance homozygous for all factors. To carry on five or six  $F_2$  plants would suffice, but in every generation some of the progeny would have to be eliminated. Elimination would have to be very carefully systematised to guard against bias, and so arranged that the critical  $F_3$  means were based on populations of sizes adequate to reliability.

Most of the work of 1920 was carried out by Mr I. G. Hamilton, a Research Student of the Empire Cotton Growing Corporation, whose help is gratefully acknowledged.

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# LINKAGE IN THE SWEET PEA (*LATHYRUS ODORATUS*).

By R. C. PUNNETT, F.R.S.

(With Six Text-figures and Plate III.)

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## INTRODUCTION.

THE data discussed in this paper have accumulated in the past 19 years. During the earlier part of this period, 1904-1909, Mr Bateson and I were working more especially upon what we then termed "coupling and repulsion" in sweet peas. The discovery of groups of characters shewing this phenomenon of linkage suggested that a further study of the subject was likely to be of interest in connection with the much discussed possibility of the location of genetic factors in the chromosomes. Though we failed to devise any explanation of linkage in terms of chromosomes, and eventually put forward the hypothesis of "reduplication," we felt nevertheless that a thorough analysis of the sweet pea ought to be undertaken. The object of this analysis was to decide whether the number of characters, or groups of characters, shewing independent inheritance was greater, equal to, or less than the haploid number of the chromosomes.

At the time we decided to undertake this analysis we had, in addition to the characters belonging to the two linkage groups already found, several others of which the simple recessive nature had been demonstrated. And we knew also of further characters which promised to be of assistance in the undertaking. Moreover we had ascertained from Mr R. P. Gregory that the haploid number in *Lathyrus* was almost

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certainly 7, a point eventually confirmed by Winge in 1918. In 1909 Mr Bateson left Cambridge, and the continuation of the sweet pea work devolved upon me. Soon after this the advent of *Drosophila* brought a definite answer to the question we had set out to attack. The brilliant researches of Morgan and his colleagues shewed beyond a doubt that the number of the linkage groups was in this case the same as the haploid number of the chromosomes. Though for many the matter appeared to be finally settled, I decided to carry on with the programme of the sweet pea work, especially as it seemed at one time not unlikely that the number of independent groups of characters might prove to be greater than that of the chromosomes. But during the past summer several fresh linkages were discovered, and although the number of apparently independent groups at present stands at 8, the data available are not in all cases sufficient to preclude the possibility of a low grade of linkage\* between certain of them. Some of these I expect to test further in the near future; and I have also started experiments with other characters which I hope to work into the general scheme. Some years must, however, elapse before I can accumulate the necessary data, and I feel that the present juncture is a convenient one for taking stock of the position. Though the question we set out to answer cannot be regarded as finally settled, I have nevertheless come to the opinion that the number of linkage groups in *Lathyrus* will eventually be found to correspond to the haploid number of the chromosomes.

### *Material.*

The account given below deals with seventeen pairs of characters shewing normal Mendelian inheritance, of which ten have already been described in earlier papers. In the following notes on these characters I have adopted a system of symbols differing from that previously used. The factors in the same linkage group are now denoted by the same letter, the separate factors being distinguished by a numeral. Thus  $A^1$ ,  $A^2$ ,  $A^3$  are three distinct factors shewing linkage with one another, while  $B^1$ ,  $B^2$ ,  $B^3$  are also three factors in the same linked system. But, so far as is known, no member of the **A** series shews linkage with any member of the **B** series. In each case the dominant character of the pair is given first.

(1) Purple,  $A^1$ —Red,  $a^1$  (=  $B$ — $b$  of earlier papers). This was one of the first pair of characters worked with. There are, of course, numerous

\* I.e. where the number of the crossovers is nearly as great as the number of the non-crossovers.

shades of purples, corresponding to each of which is a shade of red. Owing to the fact that some of the other factors used are modifiers of flower colour I have rarely made use of any but deep colours. In these the dominance of purple, as judged by the eye, is complete, and it is not possible by inspection to distinguish the homozygous from the heterozygous individuals.

(2) Long pollen,  $A^2$ —round pollen,  $a^2$  (= L—l of earlier papers).

(3) Erect standard,  $A^3$ —hooded standard,  $a^3$  (= E—e of earlier papers).

(4) Dark axil,  $B^1$ —light axil,  $b^1$  (= D—d of earlier papers). Though usually quite distinct and easily classified, the dark axil is relatively pale where the flower colour is blue or blue-red. With care, however, I have not found these cases to present any difficulty.

(5) Fertile anthers,  $B^2$ —sterile anthers,  $b^2$  (= F—f of earlier papers).

(6) Normal flower,  $B^3$ —cretin flower,  $b^3$  (= N—n of earlier papers).

(7) Tall, E—Cupid e (= T—t of earlier papers).

(8) Colour,  $F^1$ —*R*-white  $f^1$  (= C—c of earlier papers). This is the white originally found in Emily Henderson with round pollen. That association was, doubtless, accidental. In our explanation of the appearance of the reversionary purples from a cross between the white Emily Henderson with round pollen, and the white Emily Henderson with long pollen, we denoted the two postulated complementary factors by C and R. Hence the use of the terms "*R*-white" and "*C*-white" (cf. (10) below). For by "*R*-white" we denoted the white which carried the factor R, and by "*C*-white" the white, viz. Emily Henderson with long pollen, which carried the factor C.

(9) Procumbent,  $F^2$ —bush,  $f^2$  (= P—p of earlier papers).

(10) Colour,  $G^1$ —*C*-white,  $g^2$  (= R—r of earlier papers).

All of the above ten pairs of characters have already been described in one or other of Reports I—IV of the Evolution Committee to the Royal Society. The remaining seven pairs, of which brief notes are now given, have not hitherto been mentioned in the course of this work.

(11) Hairy, C—glabrous, c. This glabrous form I owe to the kindness of Mr T. H. Dipnall, who found it in his cultures and was good enough to send me some seed. The stems are quite smooth, lacking the short stiff hairs which give a rough feel to the stem of the normal sweet pea. The difference is most noticeable in the very young pods, which, in this variety, are devoid of the silky hairs so characteristic of the



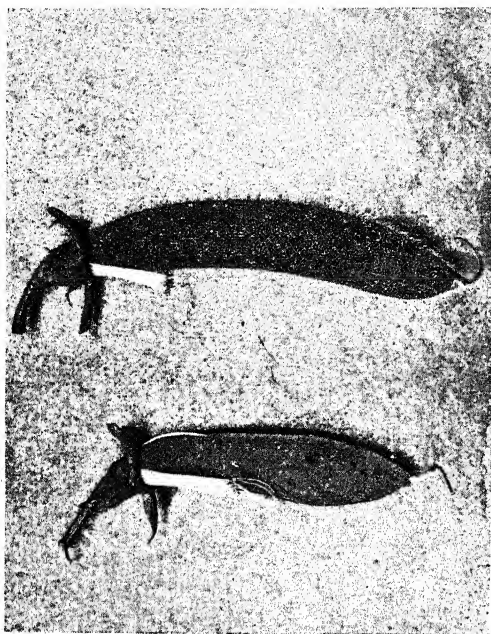


Fig. 1. Two immature pods, the upper hairy, and the lower glabrous.



Fig. 8. Small plant of form intermediate between tendrillar and acacia forms.





Fig. 2.

normal form. The glabrous character of the pod remains a striking feature throughout its growth (cf. Fig. 1). Glabrousness proved to be a simple recessive, and, being a structural feature, has proved of great service in the experiments.

(12) Tendril,  $D^1$ —acacia,  $d^1$ . The acacia-leaved sweet pea was originally found by Mr Unwin, the well known expert on sweet peas, and was first grown by us in 1909 from seed which he kindly gave us. As illustrated in Fig. 2 its peculiar feature consists in the place of the tendrils being taken by leaflets. So far as my experience goes, the lack of tendrils is complete, for I have examined considerably over a thousand of these plants without finding any that shewed a tendril. The case is of some interest as being one of the few found in *Lathyrus* in which dominance is generally incomplete.  $F_1$  plants from acacia  $\times$  tendril have always shewn an occasional leaflet replacing a tendril (cf. Fig. 2), while in  $F_2$ , besides normal tendrils and acacias, intermediates of various grades generally occur. But in all of these various intermediates I have never seen any of the tendrils beyond the basal pair transformed into leaflets. In a low grade intermediate, such as a normal  $F_1$  plant, only an occasional basal tendril is transformed: in a high grade intermediate most of the pairs of basal tendrils throughout the plant are transformed into leaflets (cf. Fig. 3). Such high grade intermediates I have found always to be heterozygotes, throwing tendrillar plants, high grade intermediates and acacias in the ratio 1:2:1. In such families the tendrillar plants nearly always shew an occasional extra leaflet. From this, and from other experiments, I think it probable that in this case we are probably concerned with two factors. Where we have the clear cut 1:2:1 ratio we may suppose that we are concerned with a factor T, Tt plants being high grade intermediates, and tt plants acacias. To explain the other grades of intermediates we may postulate the existence of a factor, I, which intensifies the tendrillar character when T is present, but has no effect upon the acacia itself. Normal pure tendrillar plants contain both T and I. Hence the low grade intermediates in  $F_1$ , as well as the range of intermediates in  $F_2$ . At this stage, however, I do not wish to insist upon this interpretation. The point of importance here is that the full acacia is always unmistakeable, and always behaves as a simple recessive, whatever the grade, or grades, of the accompanying intermediate heterozygous forms.

(13) Bright flower colour,  $D^2$ —dull flower colour,  $d^2$ . To the bright series belong the normal purples and reds, those of the corresponding

recessive dull series being blues and blue-reds. In the deeper colours the bright and dull series are perfectly distinct. A blue such as "Lord Nelson\*" is the dull form of the deep purple recorded in our earlier experiments as "Duke of Sutherland†," which itself is the hooded form of *Ppw*‡ (= purple with purple wings). Among the reds the two series are equally distinct, the bright red formerly known as "Miss Hunt§" being represented in the dull series by the type shewn on Pl. III, fig. 5. It should be mentioned that this pinkish mauve colour can be closely matched by plants which are genetically purples. For instance the  $F_1$  ex "Countess Radnor"  $\times$  "Barbara" is not dissimilar, though both of these belong to the bright series, and "Countess Radnor" is genetically a purple. But such cases of resemblance are only to be found among the paler forms of purple: in the deeper colours, which alone have been made use of in these experiments, the distinction between the bright and the dull series is never in doubt.

(14) Self-coloured,  $F^3$ —marbled,  $f^3$ . Corresponding to each self-coloured form is a recessive one in which the colour is broken up by finely divided white "marbling." The general appearance of these marbled forms is well shewn on Pl. III, figs. 1—4, which represent the marbled forms of blue, red, deep purple, and blue-red respectively. The blue marbled (Fig. 1) corresponds closely with the horticultural variety known as "Helen Pierce," in which form the marbled character was originally introduced into these experiments. The marble form must not be confused with the flaked sweet peas such as "Senator" or "America." The older flowers on a marbled plant often display coarser and more blotchy markings, which can be closely paralleled by flowers from the flaked varieties. There is however a constant point of difference between the two, for in a marbled form the keel and the under surface of the wings are white, while in a flaked form these structures always shew some colour, at any rate in the darker shades with which alone I have worked.

Another peculiar feature of the marbled form is that it always shews the light axil. It can, however, carry the dark axil, as I have proved by a series of appropriate crosses. In this respect it resembles the two forms of white ((8) and (10)).

An interesting feature in the genetics of marbled is its relation to

\* Figured in *Journal of Genetics*, Vol. XII, Pl. XXI, fig. 3.

† Figured in Bateson's *Mendel's Principles of Heredity*, Pl. V, fig. 8.

‡ Figured in *Mendel's Principles of Heredity*, Pl. V, fig. 7.

§ Figured in *Mendel's Principles of Heredity*, Pl. V, fig. 9.

*R*-white. The three forms, self-coloured, marbled, and *R*-white must be regarded as forming an allelomorphic series. Marbled  $\times$  *R*-white has always given a marbled  $F_1$ , even when the *R*-white used came from a family in which all the coloured members were self-coloured. Again, self-coloured plants that throw *R*-whites do not throw marbled, while those that give marbled do not give any *R*-whites. It is the only example of what has been termed a multiple allelomorphic series that I have hitherto met with in the sweet pea.

When crossed with *C*-white, extracted from self-coloured, the marbled form gives a self-coloured  $F_1$ , and the  $F_2$  generation contains both marbled and whites. A further point of interest is found here, in that the marbled which throws *C*-white is paler than the homozygous marbled form. This would appear to be another instance of a definite heterozygous form in the sweet pea (cf. p. 106).

(15) Purple,  $G^2$ —red-purple,  $g^2$ . This pair of characters has been dealt with at length in an illustrated account recently published in Vol. xh. of this Journal. Some further notes in connection with it are given under (17) below.

(16) Clamped keel, *H*—open keel, *h*. The clamped keel is the ordinary form (Fig. 4*a*) found in the wild sweet pea and in all forms with the non-waved type of standard, whether it be erect or hooded.

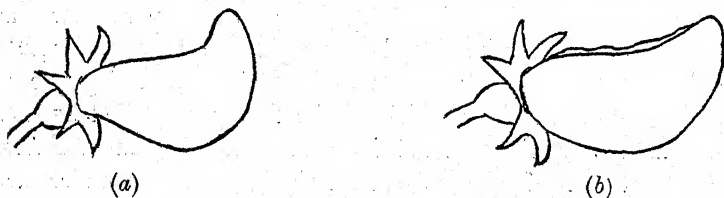


Fig. 4. Diagrammatic representation of clamped keel (a), and open keel (b).

The open keel is characteristic of the more modern type of flower with the waved or "Spencer" standard. It is markedly less curved (Fig. 4*b*) than in the clamped type; its free edges are rather waved, and not in close contact with one another; and, finally, it does not press closely against the stigma along its curvature, as is the case in the clamped form. The distinction between the clamped and open keel is in reality that between the non-waved and the waved standard. The waving of the standard is however subject to a great deal of variation, and it is not always easy to distinguish the waved from the non-waved by the appearance of the standard alone. I have, however, never found any

difficulty in the case of the keels, the distinction between open and clamped being quite sharp in the flower that is fully out.

(17) Purple,  $B^4$ —maroon,  $b^4$ . The type of maroon referred to here is that found in the variety "Dobbie's maroon," which was exhibited as a seedling at the Chelsea Show of 1919. In my experience it behaves as a recessive to deep purple. It is of interest in connection with the Purple-red-purple pair, as will appear from the following notes. After I had obtained a pure strain of red-purple, I crossed it among other things with "Robert Sydenham," to test for possible linkage in the shape of the keel. In the  $F_2$  generation, in 1918, appeared the "Spencer" form of red-purple. It was a novel and striking form, and I decided to fix it in the normal way, by growing on an  $F_3$  generation in 1919. It was therefore interesting to find Messrs Dobbie and Co. bringing out as a novelty in that year what appeared to be a form identical with that which had arisen in the course of my experiments. In 1920 I grew "Dobbie's Maroon" side by side with my own Spencerised red-purple. The growth in "Dobbie's Maroon" was a trifle more free, the stems, pedicels, and foliage a shade less dusky, and the flower-colour not quite so fiery. But the differences were slight, and the two forms would almost certainly have been regarded by experts as "too much alike" varieties. Yet genetically they are totally distinct, though I did not find this out until later. In 1920 a cross was made between "Dobbie's Maroon" and violet\*, which is the dull (or "blue") form of red-purple. Had "Dobbie's Maroon" been genetically similar to red-purple, this cross should have given a red-purple in  $F_1$ , and in  $F_2$  red-purples and violets in the ratio 3:1. Actually the  $F_1$  plants were normal purples, though with a distinctly reddish hue. In  $F_2$  appeared, besides red-purples and violets, various shades of purple, including some like the  $F_1$  plants, and some ordinary hooded purples similar in shade to the old-fashioned "Duke of Sutherland." There also appeared some normal blues. The  $F_2$  generation was a small one, some 50—60 plants only, and the analysis has not been pushed further. It is clear however that maroon and red-purple, though practically identical in appearance, differ from the normal purple in distinct factors. I have also found that from "Dobbie's Maroon"  $\times$   $R$ -white some creams appear in  $F_2$ , though I have never met with these from red-purple crossed with either  $C$ -white or  $R$ -white. Maroon is probably rather a duller form than red-purple, but in "Dobbie's Maroon" the yellow plastids of the cream basis brighten it up, so that it comes to look

\* Illustrated on Pl. XXI, fig. 2 in Vol. xii. of this Journal.

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very like a red-purple with colourless plastids. The genetical distinction between maroon and red-purple is further emphasised by the fact that they belong to different linkage systems. For red-purple shews linkage relations with the *C*-white, while maroon is linked with axil-colour.

### *Linkage Groups.*

So far five different linkage groups have been definitely detected, and the evidence for their existence may now be given.

A. Under this letter are collected the three pairs of characters: Purple-red ( $A^1$ ), long-round pollen ( $A^2$ ), and erect-hooded standard ( $A^3$ ), for the linkage relations of which evidence has already been given in earlier papers\*. That between  $A^1$  and  $A^3$  is very close, in the neighbourhood of 1% of crossovers. The number of crossovers between  $A^1$  and  $A^2$  is about 12% (cf. Haldane, 1919, p. 294). Though there is little evidence one way or the other, it seems probable from such as exists, that  $A^1$  lies between  $A^2$  and  $A^3$  on the chromosome†.

B. In this chromosome are found the factors for the dark ( $B^1$ ) and light axil pair, for the fertile ( $B^2$ ) and sterile anther pair, and for the normal shaped flower ( $B^3$ ) and cretin pair. The data for these linkages have already been given elsewhere (*Journal of Genetics*, III. pp. 84 seq.), and it is probable that  $B^2$  and  $B^3$  are about 25 units apart on the chromosome, while  $B^1$  lies about 6 units from  $B^2$ , between the latter and  $B^3$ ‡. That the purple-maroon pair is also found in this chromosome is evident from the following data. Maroon with light axil was crossed with *R*-white carrying purple and dark axil.  $F_1$  was dark axilled purple, and the  $F_2$  generation (No. 50/22) consisted of

Purple dark axil	...	99
„ light axil	...	19
Maroon dark axil	...	9
„ light axil	...	28
White	...	48

The figures point clearly enough to a linkage between maroon and light axil, but the numbers are too scanty and irregular to fix its value. Further experiments, too, are required before it is possible to fix the position of maroon in the B chromosome.

\* The most recent statement of this case will be found in *Journal of Genetics*, Vol. vi. p. 185 seq.

† See Bridges, 1914, p. 528, and Punnett, 1917, p. 189.

‡ Bridges (1914) arrives at rather different values, but the point is here immaterial.

D. In 1919-20 a number of families from the cross between bright flowered acacia ( $d^1D^2$ ) and dull (blue) flowered tendril ( $D^1d^2$ ) gave bright flowered tendrils in  $F_1$ . The  $F_2$  generation comprised a number of families distributed as follows among the four expected classes:

Bright flowered tendril	...	847
Dull                   "                   "	...	298
Bright flowered acacia	...	300
Dull                   "                   "	...	49

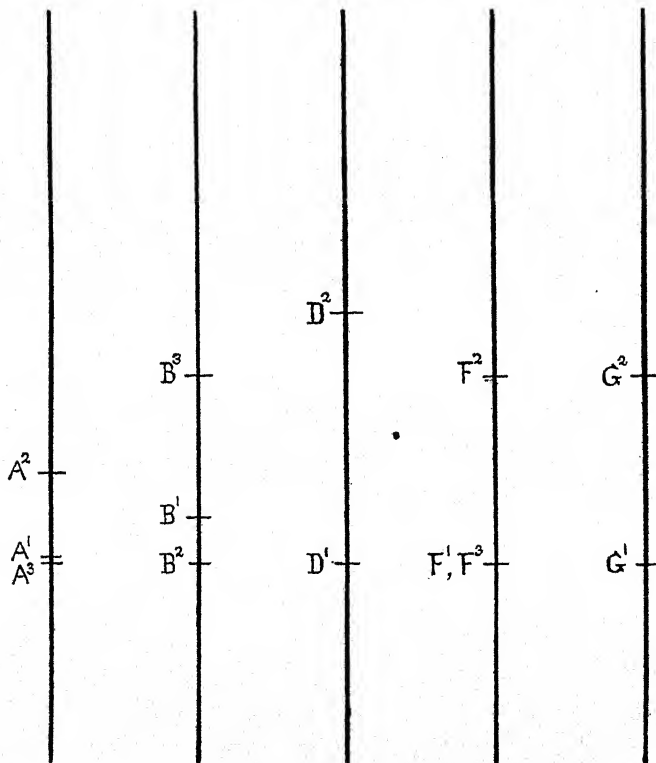


Fig. 5. Provisional map of the chromosomes for the five linkage groups hitherto identified. The figure shews only the relative, not the actual position on the chromosomes.  $B^2$  for example may be either near the middle, or at the extreme end of the chromosome. Further data are required before the positions can be fixed more accurately.

The deficiency of the dull acacias suggested the possibility of a linkage between bright colour and acacia. Accordingly the reciprocal



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cross between bright tendril and dull acacia was made.  $F_1$  was bright tendril as before, but  $F_2$  consisted of

Bright tendril ...	424
Dull „ ...	99
Bright acacia ...	102
Dull „ ...	91

Evidently there is linkage, and the figures suggest that the number of crossovers is somewhere in the neighbourhood of 33 %.

F. That *R*-white and marbled must, on the chromosome hypothesis, occupy the same locus, has already been pointed out. There is also evidence of linkage between *R*-white and bush. In 1905 a cross was made between the bush sweet pea and the ordinary procumbent cupid, both being white flowered. As recorded elsewhere (*Reports to the Evolution Committee of the Royal Society*, iv. p. 6),  $F_1$  was tall purple. In  $F_2$  appeared the expected segregation, bush cupids appearing as well as bush tall, while the ratio of coloured to whites was 9:7. Other experiments shewed that of the two parents the bush was to be regarded as the *C*-white, and the cupid as the *R*-white. The cross was therefore of the nature  $g^1F^1f^2$  (bush)  $\times$   $G^1f^1F^2$  (cupid). The  $F_2$  generation consisted of the four following classes:

Coloured procumbent ...	130
„ bush ...	53
White procumbent ...	89
„ bush ...	24

The bush plants formed more than a quarter of those with coloured flowers—less than a quarter of those with white flowers.

This peculiar distribution was confirmed in the  $F_3$  generation, where 4 families from parents heterozygous for both of the colour factors gave the following results:

		Coloured procumbent	Coloured bush	White procumbent	White bush
1908	No. 133	83	57	101	17
	„ 135	161	70	141	36
	„ 139	66	26	42	9
	„ 159	57	21	60	9

If it be supposed that bush and *R*-white shew linkage, and that the number of crossovers is about 25 % (= repulsion on a 3:1 basis between



$F^1$  and  $F^3$ ), then we should expect the four classes to appear in the ratio 99 : 45 : 93 : 19. For the  $F_2$  and  $F_3$  results the combined figures are :

	Coloured procumbent	Coloured bush	White procumbent	White bush
	497	227	433	85
<i>Expectation</i>	485	218	447	92

and they are evidently in close accord with linkage of the value suggested.

In addition to the four  $F_2$  families recorded above, in which the ratio of coloured : white was 9 : 7, there were four others in which the ratio was 3 : 1, viz. :

		Coloured procumbent	Coloured bush	White procumbent	White bush
1908	No. 128	29	8	6	3
	„ 129	20	3	2	2
	„ 158	48	17	23	1
	„ 161	174	66	69	6

In the last two families (Nos. 158 and 161) we again meet with the phenomenon of an excess of bush plants among the coloureds, and a deficiency among the whites. If we suppose that these  $F_2$  parents were heterozygous for  $R$ -white, and homozygous for the other colour factor, and at the same time were produced by the gametic union  $F^1f^2 \times f^1F^2$ , the numbers are close to what would be expected on a 25% crossover basis, as the following figures shew :

	Coloured procumbent	Coloured bush	White procumbent	White bush
158 } 161 }	222	83	90	7
<i>Expectation</i>	208	94	94	6

The other two families (Nos. 128 and 129) are probably of a different nature, for they shew neither excess of coloured bush, nor deficiency of white bush. It is reasonable to suppose that the whites here are  $C$ -whites, for there is an equal chance of the two kinds of white being found in  $F_3$  families, which throw coloured and white in the ratio 3 : 1. If so, of course we should look for a normal 9 : 3 : 3 : 1 ratio.

Further experiments have been started in connection with the linkage relations of the factors  $F^1$ ,  $F^2$  and  $F^3$ . Meanwhile we may assume that  $F^1$  and  $F^3$  occupy the same locus, and that  $F^2$  is about 25 units distant from this locus.

G. In 1920 a red-purple was crossed with a strain of  $C$ -white known to be homozygous for normal purple.  $F_1$  plants were normal purples,

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and in 1922 an  $F_2$  generation was raised from 14 similarly bred  $F_1$  plants. In respect of the two pairs purple—red-purple and colour—C-white, the distribution of the characters in the  $F_2$  generation was

Purple	... 156
Red-purple	... 78
White	... 80

The red-purple class was half the size of the normal purple-class instead of forming only one quarter of the coloured plants. Since the cross was of the nature  $G^1g^2 \times g^1G^2$ , this result is readily explicable on the assumption that linkage occurs. On these data alone however it is not possible to form any trustworthy estimate of the value of the linkage. For the following reason, however, I am inclined to think that it is not very close. As stated elsewhere (*Journal of Genetics*, XII. p. 256) the red-purple differs in habit from the corresponding normal purple, being smaller, and at the same time "duskier" in appearance. Though of course the duskiness is absent in whites, I have noticed that in an  $F_2$  generation from  $R$ -white  $\times$  red-purple, some of the whites recall the red-purple habit. The foliage is of a rather darker green, and in well-grown plants it is possible, with care, to separate the two kinds. The  $F_2$  generation from  $C$ -white  $\times$  red-purple was not well grown, owing to the late germination of most of the seeds, and I did not attempt to distinguish "red-purple"  $C$ -whites from the rest. Nevertheless I noticed several plants which gave me the impression of belonging to the "red-purple" class. As the total  $F_2$  generation comprised only 314 plants, the presence of 5 such plants would indicate a crossover value of about 25%. I am inclined to think therefore that it lies somewhere about here, but until further experiments, now in progress, have matured, the point must remain unsettled. I do not however feel any doubt of the existence of linkage between these two pairs of characters.

*The linkage-testing data.*

Of the pairs of characters used in this work, three, viz.  $C$ ,  $E$ , and  $H$ , appear to stand outside the 5 linkage systems hitherto identified; and we may now consider the evidence for this, as well as for the independence of the 5 linkage systems themselves. Back-crosses of the doubly heterozygous individual on to the double recessive, such as have been used with such success in *Drosophila*, are impracticable in *Lathyrus*, owing to the great amount of time and labour involved in obtaining reasonably large numbers. For deciding the question whether linkage does or does not occur between two given pairs of characters, the only

practicable method is to make the cross both ways and to examine the  $F_2$  generation in each case. By making the cross "both ways" is of course meant that the crosses are of the nature  $XY \times xy$ , as well as of the nature  $Xy \times xY$ , when  $X$  and  $Y$  represent the factors corresponding to the two dominant characters of the pairs whose relations are being investigated. During the past 12 years an attempt has been made to cross nearly every one of the 16 pairs of characters given on page 116 with nearly every other one, and, so far as was possible, to make the cross both ways. The number of crosses undertaken, from which an  $F_2$  generation was raised, is shewn graphically on Fig. 6: the actual data are given in condensed form in Table I, pp. 119—122. In some cases the cross between two characters was not made because the evidence already obtained shewed it to be unnecessary. In other cases again the cross was made, but no results were obtained, either because it failed, or because the  $F_1$  plants died prematurely through disease or drought. In some cases also the  $F_2$  figures are, for similar reasons, smaller than might reasonably have been expected.

In the majority of the crosses given in Table I the figures are clearly opposed to the idea of any linkage. Some of them taken by themselves may suggest that linkage occurs, but when considered in connection with other crosses, involving other members of the same linkage groups, it will become evident that such figures are due to what may be termed accidental irregularities. To take an example—the cross  $A^2 \times F^3$ , which was made in the manner  $A^2f^3 \times a^2F^3$ , gave in  $F_2$  the expected four classes in the proportion 330:90:116:22. Here the fourth term is well below expectation, as would be expected if there were a low grade of linkage involved. But  $F^1$  is situated at the same locus as  $F^3$ , and if there were linkage we should look for this to be reflected in the  $F_2$  results from the cross  $A^2F^3 \times a^2f^3$ . The actual  $F_2$  numbers here however are exceedingly close to the expectation on a 9:3:3:1 basis. Moreover, various other crosses between the  $A$  and  $F$  groups, viz.  $A^1 \times F^2$  (both ways),  $A^1 \times F^3$ ,  $A^3 \times F^1$ , and  $A^3 \times F^3$ , give no indication of any linkage. Yet if linkage really existed between  $A^3$  and  $F^3$  we should expect to find it reflected in some of these other crosses. From the fact that it is not evident, we must conclude that the deficiency of the fourth term in the  $F_2$  generation from the cross  $A^2f^3 \times a^2F^3$  is due rather to some accident (such, perhaps, as a higher mortality among marbled plants with round pollen) than to any linkage.

For the interrelations between the five groups  $A$ ,  $B$ ,  $D$ ,  $F$ ,  $G$  the figures may be left to speak for themselves. The evidence is, I think

A'	A <sup>2</sup>	A <sup>3</sup>	B'	B <sup>2</sup>	B <sup>3</sup>	C	D'	D <sup>2</sup>	E	F'	F <sup>2</sup>	F <sup>3</sup>	G'	G <sup>2</sup>	H
A'	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
A <sup>2</sup>	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
A <sup>3</sup>	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
B'	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
B <sup>2</sup>	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
B <sup>3</sup>	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
C	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
D'	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
D <sup>2</sup>	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
E	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
F'	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
F <sup>2</sup>	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
F <sup>3</sup>	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
G'	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
G <sup>2</sup>	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
H	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

- A<sup>1</sup> Purple—red.  
 A<sup>2</sup> Long—round pollen.  
 A<sup>3</sup> Erect—hooded standard.  
 B<sup>1</sup> Dark—light axil.  
 B<sup>2</sup> Fertile—sterile anthers.  
 B<sup>3</sup> Normal—cretin flower.  
 C Hairy—glabrous.  
 D<sup>1</sup> Tendril—Acacia.  
 D<sup>2</sup> Bright—dull flower colour.  
 E Tall—cupid.  
 F<sup>1</sup> Colour—R-white.  
 F<sup>2</sup> Proeminent—bush.  
 F<sup>3</sup> Self colour—marbled.  
 G<sup>1</sup> Colour—C-white.  
 G<sup>2</sup> Purple—red-purple.  
 H Clamped—open keel.

Fig. 6. Scheme illustrating the various crosses made between 16 pairs of characters. The actual data will be found in Table I. In none of these crosses was there any definite indication of linkage.  $\times$  signifies that the cross was made both ways, *i.e.* that both of the combinations  $XY \times xy$ , and  $xy \times XY$  were made.  $\times$  signifies that the cross was only made either in one way or in the other.

sufficient to convince the critic that these five linkage groups are independent of one another; and that the factors concerned must, on the chromosome hypothesis, be regarded as situated in different chromosomes.

There remain for consideration the factors C, E, H, and these may be considered separately in connection with the figures in Table I\*.

(1) The factor C.

**A × C.** If C were about 50 units distant from A<sup>1</sup> and A<sup>3</sup>, which are close together, then if it is on the same side of the chromosome as A<sup>2</sup>, it should shew about 38 % of crossovers with A<sup>2</sup>. The figures for the A<sup>2</sup> × C cross are 311 : 96 : 121 : 33, where expectation on the 38 % crossing over basis would be 301 : 120 : 120 : 20. The actual figures lie on the whole between this expectation and the figures based on absence of linkage, and the question must at present remain undecided, though the evidence is, perhaps, more against linkage than for it.

**B × C.** The results from B<sup>1</sup> × C might be construed as representing a linkage near 50 %; for both end terms are rather below, and both middle terms rather above normal expectation. The B<sup>2</sup> × C figures are irregular, and suggest rather that there is a higher mortality among the smooths, both fertile and sterile, than any linkage. On the whole, linkage here is unlikely though the matter cannot be regarded as settled until results have been obtained from the B<sup>3</sup> × C cross.

**C × D.** The evidence here affords no grounds for supposing that linkage occurs.

**C × E.** The numbers, though small, give no indication of linkage. But, of course, a crossover value in the region of 50 % is not ruled out.

**C × F.** In the C × F<sup>3</sup> cross the fourth term is low. But on the other hand the middle terms are also below normal expectation instead of above it, as they should be if there were linkage. And the first term is well above normal expectation instead of being below it. On the whole, the figures are more in accordance with a higher mortality among the marbled classes. The results of the crosses with F<sup>1</sup> and F<sup>2</sup> offer no clear grounds for assuming the existence of linkage.

**C × G.** The numbers seem to point clearly to there being no linkage here.

\* Some years ago we suggested the possibility of linkage between the purple-red and the dark-light axil pairs (cf. Bateson and Punnett, 1906, p. 36). The figures then obtained were not, however, conclusive; and since that date the point has been more fully investigated. The data obtained since 1906 are given in Table I, and tell conclusively against the existence of such a linkage.

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$C \times H$ . The numbers, though small, are in close accordance with normal expectation, though here again a crossover value of 50 % is not ruled out.

### (2) The factor E.

$A \times E$ . The figures afford no indication of linkage; but since  $A_1$  and  $A_3$ , which alone were used, are close to one another, the possibility of a 50 % crossover is not ruled out.

$B \times E$ . The fourth term in the numbers from  $B^1 \times E$  is much larger than on normal expectation. If this be taken as indicating low grade linkage, the locus of E must be about 50 units from  $B^2$ , on the side on which  $B^1$  lies.

$D \times E$ . Though the numbers are irregular the facts are against linkage. For  $D^1$  and  $D^2$  are so far apart that if E shewed 50 % linkage with either one it would have to shew much closer linkage with the other\*.

$E \times F$ . Since  $F^2$  and  $F^3$  are some 25 units apart, and since E shews no clear indication of linkage with either, it may be taken as fairly certain that the locus of E is not in the F chromosome.

$E \times G$ . Though there is some irregularity in the numbers, there is no indication of linkage.

$E \times H$ . The numbers of the cross made do not point to linkage, but the possibility of a 50 % crossover value is not ruled out.

### (3) The factor H.

$A \times H$ . Though there is irregularity in the middle terms in the reciprocal crosses, the end terms in each case are close to expectation. The possibility of a 50 % crossover value is not ruled out.

$B \times H$ . Since  $B^1$  and  $B^2$  are only about 6 units apart the possibility of a crossover value of about 50 % is not entirely ruled out.

$D \times H$ . Since  $D^1$  and  $D^2$  are about 35 units apart, it is improbable, in view of the figures obtained, that the locus for H is in chromosome D.

$F \times H$ . In the absence of data relating to  $F^3$  the possibility of a 50 % crossover value is not ruled out.

From these considerations it is clear that for the 3 factors C, E, H there are many possibilities of a very low grade linkage, in the neighbourhood of 50 %. If only one of these should be proved the number of independent groups would be reduced from 8 to 7, which is the

\* But see p. 111.

haploid number of the chromosomes. But in order to settle this point there is need for the utilisation of further characters. The greater the number of characters dealt with, the more surely can be assigned to each its peculiar chromosome and locus. The number of workable characters in *Lathyrus* hitherto unexploited is small; for the finer shades of flower colour present difficulties in classification which render their use undesirable. Structural features are the most satisfactory to use; and I should be most grateful for assistance in the shape of seeds from any novelty, however undesirable horticulturally, that readers of this paper may happen to come across.

In conclusion I wish to express my thanks to the Director of the John Innes Horticultural Institution for facilities freely afforded for growing sweet peas at Merton, and for the beautiful drawings reproduced on Plate III, which were made by Mr Osterstock. Especially, too, am I grateful to those who have helped me, both at Cambridge and at Merton, in the labour of recording the many thousands of plants which were grown for the data in this paper.

TABLE I\*.

	Type of Mating $XY \times xy$				Type of Mating $Xy \times xY$			
$A_1 \times B_1$	1363 <i>1318.5</i>	393 <i>439.5</i>	438 <i>439.5</i>	150 <i>146.5</i>	3251 <i>3345</i>	1112 <i>1116</i>	1148 <i>1116</i>	438 <i>372</i>
$\times B_2$	3894 <i>3895</i>	1220 <i>1299</i>	1390 <i>1299</i>	422 <i>433</i>	511 <i>525</i>	186 <i>175</i>	167 <i>175</i>	69 <i>58</i>
$\times B_3$	308 <i>296</i>	84 <i>99</i>	108 <i>99</i>	27 <i>33</i>	421 <i>454.5</i>	126 <i>151.5</i>	195 <i>151.5</i>	66 <i>50.5</i>
$A_2 \times B_1$	1442 <i>1368</i>	458 <i>462</i>	399 <i>462</i>	167 <i>154</i>	493 <i>492</i>	160 <i>165</i>	170 <i>165</i>	54 <i>55</i>
$A_3 \times B_1$	203 <i>195</i>	63 <i>66</i>	70 <i>66</i>	13 <i>22</i>	409 <i>430</i>	164 <i>143</i>	142 <i>143</i>	49 <i>48</i>
$\times B_2$	241 <i>236</i>	75 <i>78</i>	77 <i>78</i>	25 <i>26</i>	691 <i>680</i>	239 <i>226</i>	217 <i>226</i>	60 <i>75</i>
$\times B_3$	—	—	—	—	145 <i>142</i>	42 <i>47</i>	45 <i>47</i>	20 <i>16</i>
$A_1 \times C$	—	—	—	—	584 <i>626</i>	211 <i>208</i>	245 <i>208</i>	71 <i>69</i>
$A_2 \times C$	—	—	—	—	311 <i>316</i>	96 <i>105</i>	121 <i>105</i>	33 <i>35</i>
$A_3 \times C$	405 <i>375</i>	112 <i>125</i>	119 <i>125</i>	31 <i>42</i>	—	—	—	—

\* The figures in italics shew the expectation on a 9 : 3 : 3 : 1 basis.

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TABLE I—continued.

	Type of Mating $XY \times xy$				Type of Mating $Xy \times xY$			
$A_1 \times D_1$	1270 1261	381 420	448 420	142 140	602 599	207 199	187 199	67 66
$\times D_2$	—	—	—	—	1778 1780	552 593	636 593	197 197
$A_2 \times D_2$	—	—	—	—	369 356	109 119	115 119	40 39
$A_3 \times D_1$	526 528	179 176	174 176	59 56	—	—	—	—
$\times D_2$	399 404	149 134	129 134	39 44	—	—	—	—
$A_1 \times E$	110 104	26 34	39 34	8 11	189 189	62 63	66 63	19 21
$A_3 \times E$	96 86.5	21 28.5	26 28.5	10 9.5	—	—	—	—
$A_1 \times F_2$	228 238	73 79	93 79	28 26	322 311	102 103	95 103	32 34
$\times F_3$	—	—	—	—	1288 1222	340 408	412 408	134 136
$A_2 \times F_1$	618 616	205 205	204 205	67 68	—	—	—	—
$\times F_3$	—	—	—	—	330 313	90 105	116 105	22 35
$A_3 \times F_1$	203 198	68 66	57 66	24 22	—	—	—	—
$\times F_3$	447 402	99 134	133 134	35 44	—	—	—	—
$A_1 \times G_2$	—	—	—	—	86 85.5	32 28.5	25 28.5	9 9.5
$A_2 \times G_1$	1206 1212	437 414	406 414	129 138	307 279	89 93	78 93	22 31
$A_3 \times G_1$	1158 1185	422 396	398 396	131 132	311 313	120 104	98 104	27 35
$A_3 \times G_2$	117 117	39 39	62 58.5	16 19.5	—	—	—	—
$A_1 \times H$	150 159	65 53	48 53	19 17	556 553.5	160 184.5	208 184.5	60 61.5
$B_1 \times C$	—	—	—	—	1107 1118	381 373	390 373	110 124
$B_2 \times C$	—	—	—	—	524 498	139 166	178 166	44 55
$B_1 \times D_1$	784 778	273 259	249 259	76 86	—	—	—	—
$\times D_2$	319 331	134 111	95 111	42 87	99 90	24 30	33 30	4 10
$B_2 \times D_1$	—	—	—	—	573 573	196 191	175 191	74 63
$\times D_2$	—	—	—	—	477 469	158 157	139 157	61 52
$B_3 \times D_1$	94 76.5	25 25.5	15 25.5	2 8.5	385 373	130 124	105 124	42 41



TABLE I—*continued.*

	Type of Mating $XY \times xy$				Type of Mating $Xy \times xY$			
$B_1 \times E$	442 446	134 148	155 148	60 49	—	—	—	—
$B_2 \times E$	—	—	—	—	88 86	22 28	30 28	11 9
$B_1 \times F_1$	—	—	—	—	225 224	—	73 74	—
$\times F_2$	144 131	37 43	38 43	12 14	—	—	—	—
$\times F_3$	—	—	—	—	602 625.5	—	232 208.5	—
$B_2 \times F_1$	—	—	—	—	225 228	74 76	78 76	28 25
$\times F_3$	—	—	—	—	472 438	122 147	157 147	30 49
$B_1 \times G_2$	189 182	58 60	59 60	16 20	240 256	81 86	109 86	29 29
$B_2 \times G_1$	725 671	218 223	180 223	68 74	151 165	61 55	63 55	18 18
$B_3 \times G_1$	—	—	—	—	32 35	10 11	13 11	4 4
$\times G_2$	—	—	—	—	54 56	16 19	26 19	4 6
$B_1 \times H$	51 47	14 15	13 15	4 5	812 778	224 359	274 259	72 86
$B_2 \times H$	—	—	—	—	537 515	162 172	166 172	51 57
$C \times D_1$	—	—	—	—	148 167	51 56	80 56	18 18
$\times D_2$	405 394	112 130	134 130	43 43	331 315	108 105	96 105	25 35
$C \times E$	—	—	—	—	54 56	17 19	23 19	6 6
$C \times F_1$	—	—	—	—	314 303	105 101	91 101	28 33
$\times F_2$	—	—	—	—	130 131	41 43	52 43	8 14
$\times F_3$	—	—	—	—	729 672	194 224	222 224	49 74
$C \times G_1$	—	—	—	—	590 545	159 182	160 182	60 60
$\times G_2$	336 315	103 105	97 105	24 35	174 176	59 58	63 58	15 19
$C \times H$	—	—	—	—	67 63	18 20	19 20	6 7

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TABLE I—continued.

	Types of Mating $X Y \times xy$				Types of Mating $Xy \times xY$			
$D_1 \times E$	—	—	—	—	118 122	38 40	42 40	17 13
$D_2 \times E$	—	—	—	—	212 204.5	80 67.5	48 67.5	22 22.5
$D_1 \times F_1$	—	—	—	—	163 176	68 58	60 58	20 19
$\times F_2$	—	—	—	—	95 93	28 31	34 31	8 10
$\times F_3$	427 403	96 134	155 134	38 45	766 732	229 244	229 244	77 81
$D_2 \times F_1$	195 201	70 67	92 89	—	—	—	—	—
$\times F_3$	1868 1768.5	552 589.5	554 589.5	170 196.5	—	—	—	—
$D_2 \times G_1$	—	—	—	—	426 410.5	142 136.5	161 182	—
$\times G_2$	—	—	—	—	526 501	157 167	155 167	52 55
$D_1 \times H$	—	—	—	—	244 201	43 67	53 67	17 22
$D_2 \times H$	—	—	—	—	67 61	20 21	18 21	5 7
$E \times F_2$	—	—	—	—	521 475	122 158	151 158	50 53
$\times F_3$	70 62	14 20	18 20	7 7	—	—	—	—
$E \times G_1$	—	—	—	—	360 360	127 120	104 120	49 40
$\times G_2$	77 59	12 19	10 19	4 6	46 47	18 16	11 16	9 5
$E \times H$	—	—	—	—	330 311.5	85 103.5	109 103.5	29 34.5
$F_2 \times G_1$	108 118	40 40	49 40	14 13	139 138.5	52 46.5	40 46.5	16 15.5
$F_3 \times G_2$	—	—	—	—	413 377	121 126	107 126	30 42
$F_1 \times H$	—	—	—	—	132 136	50 45	46 45	13 15
$F_3 \times H$	—	—	—	—	33 31	7 10	11 10	3 3
$G_1 \times H$	—	—	—	—	20 21.4	7 7.1	8 7.1	3 2.4
$G_2 \times H$	—	—	—	—	132 143	53 48	55 48	15 16

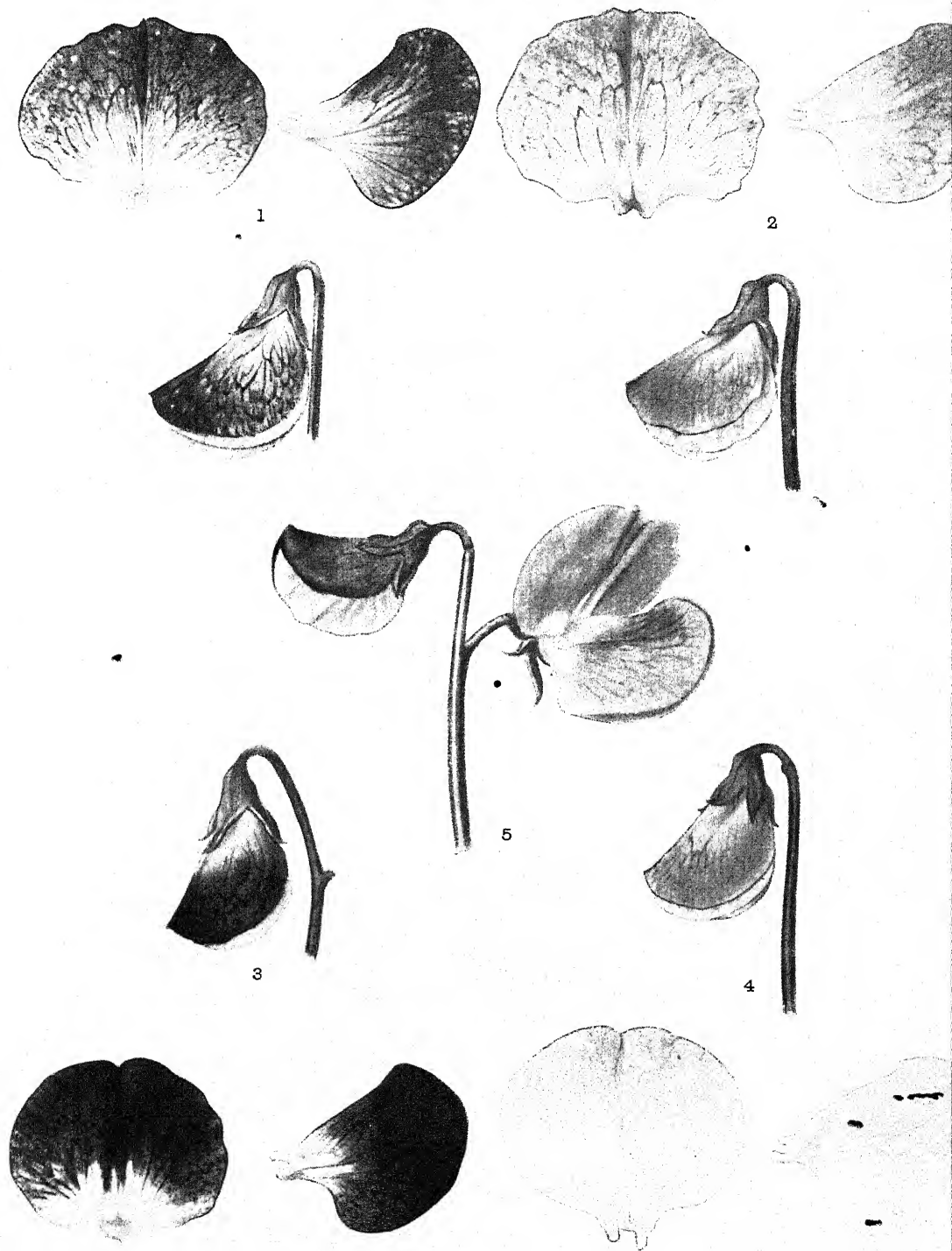
## EXPLANATION OF PLATE III.

- Fig. 1. Marbled form of blue.  
 Fig. 2. Marbled form of deep red (e.g. Miss Hunt).  
 Fig. 3. Marbled form of deep purple.  
 Fig. 4. Marbled form of blue red (Fig. 5).  
 Fig. 5. Blue-red, being the "dull" form of "Miss Hunt" in the "bright" series.

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# NEW OBSERVATIONS ON THE GENETICS OF PEAS (*PISUM SATIVUM*).

BY CAROLINE PELLEW AND ASLAUG SVERDRUP.

(*The John Innes Horticultural Institution.*)

(With Four Text-figures.)

THESE experiments relate to

- (1) The origin and properties of two new varieties.
- (2) The genetics of yellow pod, and of cotyledon colour.
- (3) Two new linkage groups.

## NEW VARIETIES.

### *Reduced stipules.*

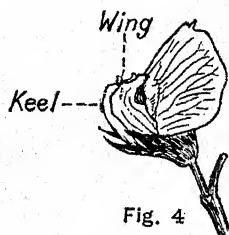
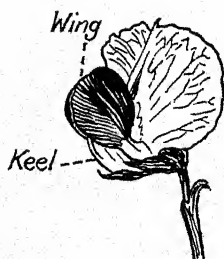
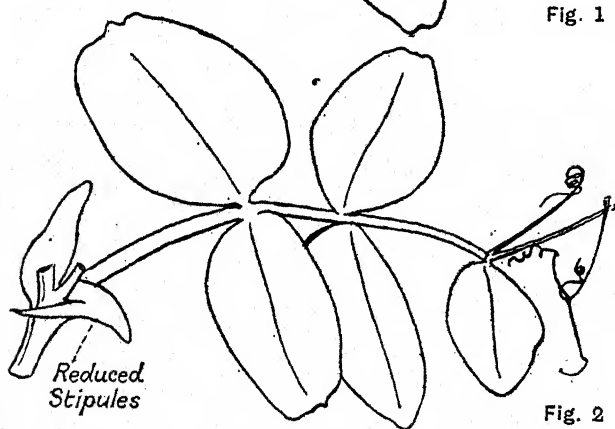
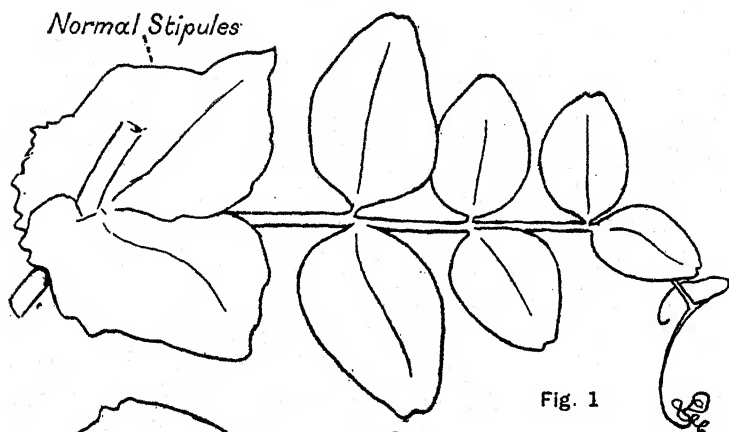
In the first variety the stipules are reduced (Fig. 2), being only a little larger than those of *Lathyrus*. It appeared spontaneously in 1915, as a single plant in a row of the variety Duke of Albany raised from seed obtained from Messrs Sutton. Messrs Sutton inform us that they have never observed a similar form. Seed from this plant gave 30 plants all with reduced stipules. Associated with the reduction in size of stipules a slight increase in size of leaflet was observed. In this family, among the green podded plants one was noticed as having *yellow pods*. A description of this and of its peculiar genetic behaviour will be given later (p. 128).

The form with reduced stipules was crossed with Duke of Albany type and rogue (Bateson and Pellew, 1915) giving  $F_1$  normal, and  $F_2$ , 3 normal : 1 reduced stipules. Crosses were also made with various other types, especially with varieties having salmon-coloured flowers, and from these,  $F_2$  families were grown showing linkage of the purple factor ( $B$ ) with that for normal stipules ( $S$ ). These families were all derived from the cross  $Bs \times bS$ , the reduced-stipuled strain being white carrying the  $B$  factor. The salmon flower colour was introduced from the fasciated form known as the "Mummy" Pea.

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*F<sub>2</sub> and comparable F<sub>3</sub> families from the cross Bs × bS.*

		Coloured				Whites	
		<i>Bs</i>	<i>bS</i>	<i>Bs</i>	<i>bs</i>	<i>S</i>	<i>s</i>
559—68. 21	...	90	37	32	2	31 : 15	
360—2. 22	...	15	5	6	—	6 : 2	
367—78. 22	...	80	28	37	1	37 : 15	
346—9. 22	...	42	24	25	1	20 : 5	
381. 22	...	9	4	4	—	none ( <i>F<sub>3</sub></i> )	
511—2. 21	...	13	7	2	—	10	1
Observed numbers		249	105	106	4	164	38
Calculated numbers		241.5	106.5	106.5	9.5		





The calculated numbers represent a gametic series of

$$1 BS : 2.5 Bs : 2.5 bS : 1 bs,$$

the cross-over classes being about 28 %.

Other allelomorphs with which *S* is not linked are as follows:

Linked with each other	{	Round—wrinkled cotyledons.
		Tendrilled—acacia leaves.
		Tall—dwarf.
		Colour—white.
		Normal habit—fasciated habit.
Linked with each other	{	Yellow—green cotyledons.
		Glaucous—emerald (free seeds).
		Glaucous—emerald (“chenille”).
		Normal wings—keeled wings.

### *Keeled Wings.*

The peculiarity of this form (Fig. 4) is that the wings have undergone homoeosis, being transformed into the likeness of the keel. Each wing stands in its normal place as a separate petal, but the tissue of which it is composed is in colour and structure exactly like the keel, being thrown up into longitudinal ridges like those of the normal keel. The rest of the flower is normal.

This form was shown to us by Mr W. A. Giles (of Messrs Sutton) in 1919. It had first been noticed (? in  $F_2$ ) among the descendants of a cross between a vetch-like rogue in *Pride of the Market* (a dwarf round-seeded variety) and the *Mummy Pea* (salmon bicolor). Keeled wings were combined with numerous characters introduced in the original cross, and several of these forms were given to us by Messrs Sutton. Since in keeled wing flowers on coloured plants the transformed wings have only the small amount of pigment characteristic of normal keels, they are not easily referred to their colour types.

Linkage of normal-keeled wing with glaucous-emerald has been tested only in the cross glaucous, keeled  $\times$  emerald, winged. The normal-winged emerald used in these crosses was the “chenille” type (*i.e.* seeds cohering when ripe) investigated by Vilmorin (1911), and Meunissier (1922)<sup>1</sup>.

<sup>1</sup> Vilmorin proved the existence of two distinct kinds of emerald, for from the cross emerald “chenille” by emerald free seeds var. “Emereva,” came a glaucous  $F_1$ , and in  $F_2$  glaucous and emerald in the ratio 9:7. We have also observed a glaucous  $F_1$  from emerald “chenille”  $\times$  emerald free seeds. From the results of this cross and from the cross glaucous free seeds  $\times$  emerald “chenille” Vilmorin showed that glaucous segregates are predominantly free seeded, and emerald segregates “chenille,” but that the “chenille” character

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Tabulated below are two  $F_2$  and six  $F_3$  families (pooled) from a cross between emerald-chenille normal wings and glaucous free keeled wings.  $K$  = normal as opposed to  $k$ , keeled wings,  $G$  = glaucous as opposed to  $g$ , emerald.

### *Families from $Kg \times kG$ .*

			$KG$	$Kg$	$kG$	$kg$
$F_2$ .	35—1. 22	...	48	31	25	1
"	522—9. 21	...	212	82	98	3
$F_3$ .	385—90. 22	...	114	51	51	1
Observed numbers ...			374	164	174	5
Calculated numbers ...			365.7	172	172	7.2

The calculated numbers represent a gametic series of

$$1 KG : 4 Kg : 4 kG : 1 kg,$$

the cross-over classes being 20%.

Other allelomorphs with which  $K$  is not linked are:

Linked with each other { Round—wrinkled seeds.  
Tendrilled—acacia leaves.  
Tall—dwarf.  
Normal—fasciated habit.  
Colour—white.  
Yellow—green cotyledons.

Linked with each other { Purple—salmon.  
Normal stipules—reduced stipules.

The linkage groups in *Pisum* of which there is now evidence are therefore three, the two here reported besides that between tendrilled—acacia and round—wrinkled seed. The other linkages which have been supposed to exist are not in our opinion authenticated. The haploid chromosome number is 7.

### YELLOW PODS AND COTYLEDON-COLOUR.

We have mentioned (p. 125) that in the family raised from our original plant with reduced stipules, a plant was noticed having yellow pods. From this plant has been raised a strain breeding true to yellow pod.

is much influenced by other characters (*e.g.* colour) and also by climatic conditions. These observations have been interpreted by O. White 1917 as indicating linkage between the two characters, but after further investigations Meunissier concludes that the "chenille" character "*est toujours corrélatif du caractère émeraude.*" Our own records of "chenille" in relation to glaucous-emerald are very limited and inconclusive. As far as we know, no case is known of plants of the cross-over combinations breeding true, and linkage of these characters remains unproven.

Subsequently we noticed that the cotyledons and testa of this particular strain are always pale yellow<sup>1</sup>. Yellowing of the pods and stems occurs during the ripening stages, the straw being creamy-yellow. This distinction is very striking under ordinary conditions, though in a wet summer the colour change is much less definite, and the plants become discoloured by moulds, so that discrimination between green and yellow pod plants is difficult and counts of mixed families are unreliable. The plants are also very slightly paler, both in stem and foliage, than the related green-podded strain even in earlier stages of growth.

The genetic behaviour of yellow pod forms has been investigated by Mendel and Tschermak (1904, p. 11). Mendel described the variety he worked with as having pods "vividly yellow, in which colouring the stalks, leaf-veins, and calyx participated." He found that this group of effects behaved as a simple recessive to green. Tschermak confirmed this result working with a variety of Sugar Pea with yellow pods.

Recent work with yellow pods has led to an extension of our knowledge of the genetics of cotyledon colour. Yellow and green, round and wrinkled, are of course the most familiar of all Mendelian characters, having been studied on an extensive scale, and ample evidence of their independence exists. O. White, however, in 1916 published observations which, as he interpreted them, suggested a definite linkage between round and a peculiar "yellow" which behaved as a recessive. In that paper he does not allude to the fact, nevertheless incidentally mentioned in his later report (1917, p. 567), that the variety used by him had yellow pods. As will subsequently appear, the suggestion of linkage is an illusion caused by the special phenomena peculiar to the yellow pod variety. As this variety is otherwise under consideration in this paper, we venture to include a preliminary note on our observations of its seed-characters though the investigation is still incomplete.

Our yellow-podded variety has wrinkled seeds. Their cotyledon colour is a *pale yellow*. This colour is not so full as that of the ordinary yellow-seeded varieties commonly used by geneticists. But in looking over samples of truly yellow-seeded peas, seeds may often occur, which could not readily be distinguished by their colour from those here under consideration. Understanding of what follows will be facilitated if we here give an outline of the results arrived at.

The pale colour characteristic of our yellow-podded variety is recessive to ordinary green cotyledons as White found in his variety. If the

<sup>1</sup> Another yellow podded plant with yellow cotyledons appeared in a crop of Duke of Albany in 1911. This plant bred true to type. We did not keep the strain.

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green-seeded variety used be a *wrinkled* (as our pale seeds also are), green is dominant, and an ordinary  $F_2$  ratio, 3 greens : 1 pale follows the pales being those which would become yellow-podded plants. But if *round*-seeded varieties are used in the cross, there is a complication from the fact that the introduction of the round character may have a direct effect on the colour of the seeds.

The simplest cross of that kind has not been made by us, but it was made by White, who found that pale wrinkled  $\times$  green round gives green round dominant. The details for  $F_2$  are not given, but the classes were three only, the *pale round* class being missing.

We crossed the pale wrinkled with an ordinary round, *yellow*-seeded variety, Mummy, which gave round yellow  $F_1$ , and in  $F_2$ , three classes not further distinguishable on inspection, "yellow" round, green round, and "yellow" wrinkled<sup>1</sup>. At first sight, since green rounds are present, one expects *green wrinkled* also, but that class is missing.

The two sets of results are obviously reconcilable if it can be supposed that the absence of *pale round* in White's experiment and the absence of *green wrinkled* in ours are both consequences of an influence of the roundness, such that the pale when round may be green. When a *wrinkled* green is used the results are normal, as we have seen.

The subsequent investigation is rendered difficult by the impossibility of distinguishing by eye the pale seeds from the yellows. Conversely there is often difficulty in distinguishing the pale seeds from greens, a difficulty which is especially met with in certain crosses. Green seeds are in many ordinary green varieties very liable to bleach, and this is notably the case with Duke of Albany. From our experience with this variety we incline to think that there is great difference in the individual plants of such sorts, and we expect that it would be possible to separate non-bleaching from bleaching strains in them. The "pale" seeds are probably a form in which the same liability to bleach exists in a higher degree. Exceptionally, wrinkled seeds from matings with the yellow-podded variety have come green when fresh, though only pale were expected, but we have noticed that in one case such seeds have bleached to the pale colour after being put away for a year.

Of the green or greenish round seeds in  $F_2$  from the cross between Mummy and the yellow-pod variety, the majority have given plants with yellow pods. Strictly according to expectation all should have

<sup>1</sup> Mummy (seeds yellow round)  $\times$  any green-podded (seeds green wrinkled) variety gives in  $F_2$  an ordinary 9:3:3:1 ratio in seed-characters.

done this, but we have no difficulty in attributing the few exceptions to the impossibility of correctly sorting such material.

It must be clearly understood that the colour of the seeds does not, as we first thought possible, depend in any way directly on the colour of the maternal pods. Any mixture may occur in pods of either colour, if the appropriate parents are used. Whether it is possible to combine ordinary *dominant yellow* seeds with yellow pods as a true-breeding form we do not know, but we incline to think that this is not possible, and that the yellow pods and the pale colour of the cotyledons are due to the absence of a single factor. There is nevertheless some reason for supposing that a complex interrelation may exist between the colours of the testa and the degree to which the pale seeds assume their proper colour.

Until we know for certain that true yellows cannot be combined with yellow pods in a pure type, a factorial representation of the way in which the several forms are interrelated can only be conjectural. We are disposed however to regard the facts as pointing to the existence of a triple allelomorphic series composed as follows: (a) true dominant yellow cotyledons associated with green pods; (b) green cotyledons also associated with green pods; (c) pale cotyledons associated with yellow pods; the three standing in a descending order. As stated above, when the factor for round seed is associated with the lowest term in the series, the seeds which if wrinkled would have been pale, are often green or greenish.

Miss de Winton has given much help with the observations here recorded.

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(FORM NO. 30.)

## INHERITANCE OF THE THREE FORMS IN TRIMORPHIC SPECIES.

By N. BARLOW.

IN 1913 (*Journ. Gen.* Vol. III. No. 1) I published the first results of experiments on the inheritance of the three forms in the heterostyled species *Oxalis valdiviana* and *Lythrum salicaria*. I hoped to be able to carry the enquiry to a definite conclusion, for the inheritance of the three distinct forms which go to make a single heterostyled trimorphic species, seemed particularly amenable to a simple Mendelian solution. Moreover within one such species occur unique phenomena of partial and complete self-sterility and inter-sterility, the appearance of a high proportion of bad pollen grains and bad seed in certain cases:—I had hoped to make clear the connection of these phenomena with the main problem of the inheritance of the three forms. Since 1913, however, the work has been carried on intermittently. The present paper records such numbers as have been obtained, with a discussion of a schematic explanation of the results.

Dimorphic plants are recorded in several Natural Orders: Boraginaceae, Cordiaceae, Erythroxylideae, Gentianeae, Hypericineae, Linaceae, Oleaceae, Polemoniaceae, Polygonaceae, Primulaceae, Rubiaceae, Thymeliae, Verbenaceae.

Trimorphic species are only found in Geraniaceae, Lythraceae and Pontederiaceae [4], as far as I know. It may be as well to state briefly the physiological peculiarities of these plants, and to recapitulate the established facts with regard to their genetic behaviour.

In dimorphic species, there is one pollen tier to each form, agreeing approximately with the length of the style in the opposite form. But the degree of variability in length of the male and female sexual organs is far greater than was originally supposed, as Tischler has shown [8], thus weakening the case of those to whom the theory of adaptation was sufficient.

Not only are the stamens of the two forms distinguished by their length or place of insertion in the corolla tube, but also by the actual size of the contained pollen grains, those belonging to the long stamens

and Short-styled plant being in practically all cases larger than those from the short stamens of Long-styled plants [4, p. 249].

In trimorphic species, each of the three forms has two distinct pollen types, borne on stamens of different heights. The pollen differs in size according to the stamen length, and as in dimorphic plants, the long pollen is the largest.

Mr Rowbotham kindly made some measurements for me of the relative sizes of the pollen grains in *L. salicaria*. His results were in agreement with those of H. Müller, quoted in *Forms of Flowers*, p. 143. The long pollen of both forms is larger than the Mid, and the Mid is larger than the short pollen. He found the greatest divergence between the long pollen of the Short-styled, and the short pollen of the Long-styled plants, the ratio being 100:56.6.

He also made a few observations on the presence of bad pollen grains. In one large family of illegitimate offspring ( $M \text{♀} \times \text{own pollen}$ ), some individuals had normal good pollen, whilst others had a large percentage of bad grains, and grains irregular in size. In another Mid of this family ( $1^6/18$ ) he found the long pollen irregular, whilst the short pollen was normal in size and regular. Clearly segregation of some sort has taken place connected with the sterility problem. Unfortunately these promising indications have not so far been followed up. A further example of segregation is given by Darwin [5]. From an illegitimate union of Mid  $\text{♀} \times$  Short  $\text{♂}$  of Long-style, 17L and 8M were raised. Five of these were observed for fertility, and two proved "moderately sterile, and three fully fertile." Not one of this family was dwarfed.

There is a further visible difference in *L. salicaria* in the colour of two of the pollen types from the four others. Long pollen is of a varying intensity of green, whilst Mid and Short pollen is pure yellow. Exceptions have been observed to this colour differentiation.

One other point may be mentioned here, as I have never seen any reference to it. The different tiers of stamens are borne by separate whorls; the inner whorl is always the longer. It therefore follows, that though long stamens are always carried by the inner whorl, and short stamens by the outer whorl, mid-length stamens are on whorls of different origin in the two forms; the Mid stamens of a Short-styled plant are on the outer whorl, and the Mid stamens of the Long-styled plant are on the inner whorl.

So much for outward differences. For factorial differences, neither the evidence of others nor my own, has ever shown that there has been any segregation of the characters for Long, Short or Mid-style in the



two pollen types of one plant, as might easily have been anticipated. But there has been segregation of another sort; the two ♂'s of one plant differ in their compatibility with the same ♀. Thus the mid-pollen of a Long-style sets a full complement of seed on Mid-style ovules, whereas the short pollen of the same Long-style plant, will set few, or more probably none. At some period in cell-division the two tiers of pollen have become differentiated; though of the nature of this differentiation we have no clear conception as yet. In this connection Stevens's work on the growth of pollen tubes in heterostyled plants is of interest [9]. After legitimate pollination in *Lythrum salicaria*, she found in 18 hours regularly a three-celled embryo, with at least three nuclear divisions in the endosperm. The result was the same in each form, in spite of the difference in the distance travelled by the pollen tubes. After illegitimate pollination very little growth of the pollen tube had been made in 24 hours. After three days in a very few cases, the tube had extended almost to the egg. In a few flowers, investigated 96 hours after pollination, an 8- or 16-celled embryo was found.

The pollen tube presumably draws its nourishment from the tissues of the style, and it is conceivable that development is only possible in pollen and styles of the same height owing to the formation of compatible chemical substances, as suggested by Jost. If the question of self-sterility could be attributed solely to chemical incompatibility between the tissues of the style and the pollen tube, we might expect that in the rare cases where ♂ and ♀ unite, zygote development would proceed normally. But the higher mortality in the seed-box (compared with that seen in the germination of legitimate seed) and the stunted form of illegitimate plants, even after years of growth, show that the lethal factor, or inhibitor, has not ceased its operation at the union of the germ-cells.

The question of self-sterility is further complicated by the different degrees obtaining in the various crosses. Darwin found that legitimate fertility also varied according to the form of the mother used. In plants setting seed spontaneously he found the following relationship existed:

Form	Average seed per capsule	
	Count I	Count II
Long	93	80
Mid	130	97
Short	83	61

From artificially fertilized capsules he found the same greater fertility of the Mid-styled plant.

The following table contains the legitimate and illegitimate crosses.

TABLE I.

Explanation of lettering used. The capital letter denotes the form of parent, the small type the height of pollen. Thus

$L \times lS$  = Long-styled ♀ × Long ♂ of Short-style plant.

Cross	Number of flowers fertilized	Percentage of flowers setting seed	Average seed per capsule	Cross	Number of flowers fertilized	Percentage of flowers setting seed	Average seed per capsule
$L \times lM$	13	38	51.2	$M \times lS$	15	93	69.5
$L \times lS$	13	84	107.3	$M \times sL$	13	54	47.4
$L \times mL$	15	20	12	$M \times sM$	12	0	0
$L \times mS$	12	only 1 set	20	$S \times sL$	12	83	81.3
$L \times sL$	15	20	5	$S \times sM$	13	61	64.6
$L \times sM$	14	only 1 set	3	$S \times mL$	10	only 2 set	18
$M \times mL$	12	92	127.3	$S \times mS$	10	" "	15
$M \times mS$	12	100	108	$S \times lM$	10	0	0
$M \times lM$	12	33	75	$S \times lS$	10	0	0

Thus the Mid not only sets a much larger number of seed when legitimately fertilized than do Longs and Shorts, but also Mids illegitimately crossed with the two long pollens, and also with the short pollen of Longs, set a fair number of seed.

To summarize our incomplete knowledge of the inter-fertility and inter-sterility factors, we find:

1. The seed maximum per capsule differs in the three forms, Mids giving most, and Shorts least. This is true both in legitimate and illegitimate fertilizations.

2. In the 12 more or less sterile illegitimate combinations out of the 18 possible unions within the species, we get a series of possibilities—complete absence of zygote development; a much reduced seed formation, increasing to a fair percentage of the normal. Of the seeds so formed, germination is bad, mortality of the seedlings is great, and the plant usually does not attain normal height in growth.

3. The gamete development of the plants so raised is not normal, the pollen having a large percentage of bad grains.

4. However from one illegitimate fertilization ( $M \times lM$ ) there was a good percentage of seed per capsule, good germination, and quite a third of the offspring were normal in size. But many showed their illegitimate origin by bad pollen—though in some the inhibitor was apparently left out.

5. Both in *Lythrum* and *Oxalis*, illegitimate offspring showed a much greater tendency to vary in the length of stamen than legitimately raised plants. I have had in *O. valdiviana* a Mid-styled plant with one  $l$  stamen practically at the level of the styles: another with a  $S$  stamen

above the level of the styles; and another with four aberrant short stamens above the mid-length styles. I did not test these stamens for their factorial significance as I had done previously [1, footnote p. 55] when I found the aberrant stamen carried the factors for its new position, and not of its companions in the same whorl.

These side issues all require further investigation. They must have an important bearing on the main theme of this paper—the inheritance of the three forms; and there for the present we must leave them. Mechanically they affect greatly the obvious tests for gametic constitution, owing to the usual impossibility of selfing, and of crosses between like forms.

#### *Established Facts.*

In 1905 Bateson and Gregory [2] showed that the two forms of *Primula* were inherited in a straightforward Mendelian manner; Long-styled forms were homozygous and recessive and the heterozygous Short was indistinguishable in appearance from the homozygous Short.

Dahlgren [3] has recently published his numbers obtained in *Fagopyrum esculentum*, bearing out completely the above interpretation. He isolated the heterozygous from the homozygous Short, the one giving Shorts only when selfed, the other giving Shorts and Longs.

In trimorphic species, Darwin's Long-styled plants gave only Long-styled offspring; I have self-fertilized Long-styled plants of both *Oxalis valdiviana* and *Lythrum salicaria* and have always obtained Long-styled offspring only. The Long-styled form is therefore presumably hypostatic and homozygous.

I have already shown [1] that both in *Oxalis* and in *Lythrum* the cross Long  $\times$  Short and the reciprocal, and also the cross Long  $\times$  Mid and the reciprocal, give approximate equality of parental forms. In *Oxalis* a small number of the non-parental form did occur (from .8 to 1.7 per cent.), but this may be explained by insufficiently critical methods at the outset<sup>1</sup>.

The crosses which promised the ultimate solution of the problem were those between Mids and Shorts.

In 1921 G. v. Ubisch [10] discussed my numbers of 1913, and applies to them the following bi-factorial scheme:

$$\begin{array}{ll} \text{Long} = aabb & ; \text{ gametes} = ab . ab, \\ \text{Mid} = aaBb & \quad , = aB . ab, \\ \text{Short} = Aabb & \quad , = Ab . ab. \end{array}$$

<sup>1</sup> I cannot explain Hildebrand's results [6]. He grew seed from the six legitimate unions of *O. valdiviana* and obtained all three forms from each union.

It follows that  $L \times M$  will give  $1L:1M$ ; also that  $L \times S$  will give  $1L:1S$ .  $M \times S$  will give  $ab.ab, ab.Ab, aB.ab, aB.Ab$  or  $1L:1M:2S$ , a well-established result in *Lythrum* and *Oxalis*. One Short will be of the original constitution  $Aabb$ , but the second will be  $AaBb$ ,  $A$  being "grösser" than  $B$ . V. Ubisch further considers the cross Mid  $\times$  Short, where the new type of Short is used. Mid ( $aB.ab$ )  $\times$  Short ( $AB.Ab.aB.ab$ ) will give

$1L(ab.ab):3M(1aB.aB, 2aB.ab):4S(1AB.aB, 2AB.ab, 1Ab.ab)$ , which ratio has been obtained, both in my previous numbers, and in the Tables to follow. I had arrived at a similar explanation of the results, but some obvious difficulties stood in the way, and I hoped to remove these by experiment before publishing the following results. But as the few experiments on *Oxalis* which I was able to do this year may still not remove the objections to be discussed, it seemed best to publish the present paper without further delay.

The chief obstacles to v. Ubisch's hypothesis are as follows:

1. The derived Short, of constitution  $AaBb$  which will have gametes  $AB.Ab.aB.ab$ , when crossed with a Long ( $ab.ab$ ), should give the ratio of  $1L:1M:2S$ . As already stated,  $L \times S$  and  $S \times L$  have always only given Longs and Shorts. It is true that the Short which on the above hypothesis should give all three forms when crossed with a Long, will be much more rarely met with than the normal type, but I cannot believe this a sufficient explanation.

A further assumption that in the derived Short  $AaBb$ ,  $A$  and  $B$  are completely linked in gametogenesis, will remove the difficulty. No Mids should appear in the cross  $AB.ab \times ab.ab$ . But where now is our  $1:3:4$  ratio? A Mid  $\times$  with the derived Short will now be represented by

$$M(aB.ab) \times S(AB.ab) \\ = 1L(ab.ab):1M(aB.ab):1S(AB.ab)+1S(AB.aB).$$

Again

$$1L:1M:2S.$$

But we cannot have it both ways; we cannot explain the  $1:3:4$  ratio of  $Ab.aB \times aB.ab$ , which assumes the formation of gametes

$$AB.Ab.aB.ab,$$

and at the same time eliminate the difficulty that such a Short must give Mids when crossed with a Long.

2. The second difficulty applying to the simplest bi-factorial hypothesis is that an occasional Mid  $aB.aB$  should be met with, giving Longs only when crossed with a Long, another result never obtained.

3. The *Oxalis* numbers of my previous paper are still unexplained.

4. The conflicting evidence of single cases given below.

Thus clearly the hypothesis requires further emendation, and until we get more experimental fact, the scheme must stand on an admittedly weak foundation.

*Numbers obtained in Lythrum salicaria.*

All the following families are derived from two original plants, Parent I (Short-style) and Parent II (Mid-style)

Cross I. *S* (Parent I) ♀ × *M* (Parent II) ♂ gave 32*L*:27*M*:73*S*,

„ II. *M* (Parent II) ♀ × *S* (Parent I) ♂ gave 25*L*:29*M*:66*S*.

Added together we get 57:56:139, or a near approximation to 1:1:2 (calc. = 63:63:126).

Mids from cross I were crossed back with Short Parent I.

TABLE II.

Mid ♀ × Short ♂	Gave	Long	Mids	Short
1/2 × I	„	19	23	41
1/4 × I	„	2	4	11
1/8 × I	„	26	38	62
1/9 × I	„	7	14	24
1/12 × I	„	1	5	3
1/19 × I	„	6	5	4
1/23 × I	„	5	4	10
1/24 × I	„	13	18	43
1/25 × I	„	34	27	39
<hr/>				
Short ♀ × Mid ♂				
I × 1/25	„	23*	17	41
„	„	14*	20	34
„	„	14*	14	31
„	„	2*	1	0
„	„	3*	5	10
<hr/>				
Totals		56	57	116

\* All crosses marked with an asterisk were made in 1918, and every precaution was taken to avoid error. Families without an asterisk cannot be considered as critical. The females of these asterisked families were emasculated, and the unused tier of pollen was removed from the bud of the male parent. This had not been considered necessary in *Oxalis valdiviana* owing to the much greater self-sterility of this plant. The germination of all 1918 seed showed great improvement over that of previous years. The method was adopted of sowing very lightly in the autumn and submitting to frost.

The grand total of Short I × offspring Mids is 169 Longs, 195 Mids, 356 Shorts (calc. 180:180:360). In other words Short I is *Aabb*, and all the Mids are *aaBb*.

The Shorts derived from cross 1 were crossed back with Mid-Parent II.

TABLE III.

Short ♀ × Mid ♂	Gave	Longs	Mids	Shorts
1/3 × II	„	2	32	29
1/5 × II	„	1	1	4
1/14 × II	„	0	1	3
1/18 × II	„	2	4	9
<hr/>				
Mid ♀ × Short ♂				
II × 1/1	„	15*	17	42
„	„	16*	23	45
	Totals	31	40	87
<hr/>				
II × 1/3	„	12*	29	22
„	„	8*	28	41
	Totals	20	57	63
<hr/>				
II × 1/5	„	5*	25	33
„	„	7*	35	36
	Totals	12	60	69
<hr/>				
II × 1/13	„	7*	25	37
„	„	10	20	18
„	„	7*	24	26
	Totals	24	69	81
<hr/>				
II × 1/14	„	5*	17	26
„	„	10*	24	63
„	„	11*	48	35
	Totals	26	89	124

\* See footnote p. 139.

The total of Mid II crossed with derived Shorts is 118 Longs: 353 Mids: 469 Shorts. Probably however these should not all be classed together. If we take 1/1 as being a Short of constitution *Aa.bb*, and all the others as being *AaBb*, then we get:

Mid (*aaBb*) × Short (*Aabb*) give 31*L*:40*M*:87*S*  
(calc. 39.5*L*:39.5*M*:79*S*),

and Mid (*aaBb*) × Short (*AaBb*) + reciprocals  
give 87*L*:313*M*:382*S* (calc. 98*L*:294*M*:392*S*).

Thus of the six Shorts chosen at random from the original cross, only one was *Aabb*, and five were *AaBb*. We should expect the latter to be in the minority so that the occurrence of five in six is contrary to expectation, and rather a large deviation to be accounted for by chance.

A further point to note in Table III is in the cross  $II \times 1/3$ . In 1914  $1/3 \times II$  gave  $2L:32M:29S$ . In 1918 the reciprocal cross was made critically (see footnote) and the families raised from two separate capsules were counted separately. One gave  $12L:29M:22S$ , and the other gave  $8L:28M:41S$ . A similar discrepancy may be seen in the capsules of the cross  $II \times 1/13$ . Such results are discouraging, as they are unexplained by the given scheme. But as it is impossible to account for them by error, they must take a prominent position amongst the other established facts. It seems necessary to infer that some sort of patchwork distribution is going on in the plant, causing fairly wide deviation from flower to flower, and that only from the accumulation of large numbers will the proportion work out correct according to expectation.

A piece of disquieting evidence must now be considered.

Some few of the  $F_1$ ,  $M$ 's and  $S$ 's were intercrossed and the following numbers show that the simple scheme put forward by v. Ubisch will not work.

$S 1/1 \times M II$  gives (Table III)  $31L:40M:87S$ ,

$S 1/1 \times M 1/2$  gives  $4L:11M:15S$ .

If  $M$ 's are all similar then this should be  $1:1:2$ . But this last family is too small to cause serious anxiety. But more disquieting are the following:

$S 1/3 \times M II$  gives (Table III)  $20L:57M:63S$ ,

and  $S 1/3 \times M 1/2$  gives  $28L:30M:64S$ .

Here we get the same  $S$  giving with one  $M$  the possible  $1:3:4$  ratio, and with another the  $1:1:2$  ratio. In fact the inference seems necessary that the  $M$ 's are different, and not in the only way to be accounted for in the scheme in its simplest form; that is as  $aB ab$  and  $aB aB$ .

A further generation ( $F_2$ ) was raised from the cross  $II \times 1/3$ .

SI × MII													
		32L			27M			73S					
F <sub>1</sub>		L	M	S						L	M	S	
F <sub>2</sub>	19	23	44	I × —	1/2	1/1	— × II	31	40	87			
	2	4	11	I × —	1/4	1/3	— × II	22	89	92 = cross 14			
	26	38	62	I × —	1/8			(20	57	63 critically)			
	7	14	24	I × —	1/9	1/5	— × II	12	60	69			
	1	5	3	I × —	1/12	1/13	— × II	24	69	81			
	6	5	4	I × —	1/19	1/14	— × II	26	89	124			
	5	4	10	I × —	1/23	1/18	— × II	2	4	9			
	13	18	43	I × —	1/24								
73	61	119	I × —	1/25									

The following table gives an analysis of the families derived from cross 14, or  $F_3$ .

TABLE IV.

Parents		Offspring			Parents		Offspring			Reciprocals added together		
Short ♀ × Mid ♂		Longs	Mids	Shorts	Mid ♀ × Short ♂		Longs	Mids	Shorts	Longs	Mids	Shorts
14/4 × 14/1		18	17	52	14/1 × 14/4		6*	2	5	38	33	85
—		—	—	—	—		5*	5	10			
—		—	—	—	—		9*	9	18			
14/4 × 14/2		12	12	18	14/2 × 14/4		4	3	4	16	15	22
14/4 × 14/3		5	4	15	14/3 × 14/4		25*	16	27	92	81	152
—		20*	21	23	—		27*	21	45			
—		—	—	—	—		15*	19	42			
14/5 × 14/1		5*	26	30	14/1 × 14/5		0	4	4	5	30	34
14/5 × 14/2		—	—	—	14/2 × 14/5		14	43	60	14	43	60
14/5 × 14/3		5	20	29	14/3 × 14/5		3	3	7	18	48	84
—		—	—	—	—		10*	25	48			
14/6 × 14/1		8	7	14	14/1 × 14/6		—	—	—			
—		12*	11	22	—		—	—	—	26	21	42
—		6*	3	6	—		—	—	—			
14/6 × 14/2		—	—	—	14/2 × 14/6		5	4	11	5	4	11
14/6 × 14/3		14*	17	31	14/3 × 14/6		0	0	5	53	67	84
—		—	—	—	—		19*	20	25			
—		—	—	—	—		12*	13	15			
—		—	—	—	—		8	17	8			

\* See footnote p. 139.

Clearly the Short 14/5 is differently constituted from 14/4 and 14/6. There is no evidence of any difference in the make-up of the Mids.

Factorially represented, we therefore begin by getting a very complete representation.

Short 14/4 ( $Aabb$ ) × Mids ( $aaBb$ ) gives

$$146L : 129M : 259S \text{ (calc. } 133.2L : 133.2M : 266.5S).$$

Short 14/4 was moreover tested with a Long-style, and gave the two parental forms only.

Short 14/4 ( $Aabb$ ) × Long ( $aabb$ ) gives

$$19L : OM : 18S \text{ (calc. } 18.5L : OM : 18.5S).$$

Turning to 14/6, some doubt begins to creep in,

Short 14/6 ( $Aabb$ ) × Mids ( $aaBb$ )

gives  $84L : 97M : 137S$  (calc.  $79L : 79M : 158S$ ).

Here is a serious lack of Shorts unaccounted for, yet we are confirmed in 14/6's constitution by the cross with a Long-style.

Short 14/6 ( $Aabb$ ) × Long ( $aabb$ ) gave  $29L : OM : 22S$ .



But now we must face the contradictory evidence about 14/5. Assuming that here we have a Short of constitution  $AaBb$ , we get:

Short 14/5 ( $AaBb$ )  $\times$  Mids ( $aaBb$ )

gives  $37L : 121M : 178S$  (calc.  $42L : 126M : 168S$ ).

This is an excellent example of 1:3:4 ratio, and it only remained to prove that a Short so constituted gave Mids when crossed with a Long, to put the factorial scheme upon an experimental basis beyond any doubts. But this same Short crossed with a Long gave  $29L : 0M : 27S$ . Of course if in the Short 14/5 ( $AaBb$ )  $A$  and  $B$  were linked, then no Longs would be expected, as explained on p. 138. But then the theoretical explanation of the 1:3:4 ratio must also fall to the ground.

This conflict of evidence is a very serious implication against the theory in its simplest form as put forward by v. Ubisch, and I see no means of reconciliation at present.

As before, it must be noted in Table IV that where several capsules resulting from the same cross were sown, considerable divergence appears, in the resulting families. In the cross  $14/3 \times 14/4$  the three capsules gave together

$67L : 56M : 114S$  (calc.  $59.2L : 59.2M : 118.5S$ )

which we cannot doubt is the 1:1:2 ratio. But taken individually the capsules are

$25L : 16M : 27S$ ,  $27L : 21M : 45S$  and  $15L : 19M : 42S$ ,

the first and last of which are anomalies when considered separately.

#### *Self-fertilizations.*

The evidence from self-fertilizations, and illegitimate fertilizations, is no more completely satisfying than that already given. Yet in some respects the numbers afford strong support to the theory.

TABLE V.

Parents	Year	Longs	Mids	Shorts	
$L \times m + sL$ ... ..	—	8	0	0	} CD
$L \times \text{own } m + s$ ... ..	—	48	0	0	
$M \times \text{own } l + s$ ... ..	—	1	3	0	
$M \times lS$ ... ..	—	14	8	18	
$M \times sL$ ... ..	—	17	8	0	
$S \times \text{own } l + m$ ... ..	—	1	0	8	
$S \times mL$ ... ..	—	4	0	8	} NB
$M \times \text{own } l + s$ ... ..	—	0	3	0	
$S \times \text{own } l + m$ ... ..	—	1	1	8	
$S \times \text{own } l + m, (1/1)$ ... ..	1912	1	1	2	
$S \times lS$ ... ..	—	2	0	1	
$M \times \text{own } l + s (26/31)$ ... ..	—	3	9	0	
$M \times \text{own } s$ (long emasculated) (26/15)	1916	1	2	3	
$M \times \text{own } l (26/15)$ ... ..	1917	28	89	0	
$M \times \text{own } l (s \text{ not emasculated}) (26/15)$	1918	16	74	1	

The preceding table gives my earlier *Lythrum* numbers, and those recorded by Darwin, together with my subsequent families.

All the numbers given by Darwin are in accordance with the scheme, assuming

$$L = abab,$$

$$M = aBab,$$

$$S = Abab.$$

The illegitimate unions  $M \times S$ ,  $M \times L$ ,  $S \times L$  all give offspring according to expectation for the same legitimate unions.

$L$  selfed gives Longs only,

$M$  selfed gives  $1L : 3M$  (expectation  $1L : 3M$ ),

$S$  selfed gives  $1L : 8S$  (expectation  $1L : 3S$ ).

My own numbers are not so easily explained,  $S \times lS$  giving  $2 : 0 : 1$  could fall into the  $S(Aabb)$  giving  $1 : 0 : 3$ ; and the  $S \times l + mS$  giving  $1 : 1 : 8$  could be explained if  $S = AaBb$ . But the  $S1/1$  which gave  $1 : 1 : 2$  crossed with a Mid is a serious stumbling-block. For if it can give an  $M$  when selfed, we must assume it to be  $AaBb$ . Yet in Table II we have evidence that it is  $Aabb$  when crossed back with Mid Parent II. How this conflicting evidence can be explained I do not see.

The Mid 26/31 gave  $3L : 9M$  exactly according to expectation.

The Mid 26/15 gave with short pollen  $1L : 2M : 3S$ ; with long pollen  $28L : 89M$ , and with long and possibly some short,  $16L : 74M : 1S$ . What the presence of these Shorts means, I cannot say. Apart from them, the numbers  $45L : 165M$  agree fairly well with the expectation of  $1 : 3$  for  $aaBb$  selfed.

#### *Oxalis valdiviana*.

I cannot bring my *Oxalis* numbers published in 1913 [i. p. 59] completely into the above scheme.  $L \times M$  and  $L \times S$  and reciprocals gave the two parental forms, with a few of the third form, possibly errors.

Dividing the  $F_2$   $M \times S$  and  $S \times M$  up into groups, we get

$$118 L : 121 M : 270 S,$$

possibly  $1 : 1 : 2$ .

Also  $23L : 23M : 49S$  clearly  $1 : 1 : 2$ .

Also  $6L : 102M : 101S$ , where there were no  $L$ 's in the grand-parentage. This curious result recurred in  $F_4$ . Table VI gives a summary of the results. Reciprocals are added together. All grand-parents were  $M$  and  $S$  only.

The first result,  $M \times S = 0 : 232 : 202$  can be explained in the following manner:

$$M(aBaB) \times S(Abab) = aBAb, aBab, aBAb, aBab \text{ or } 2M : 2S.$$

The second group of results shows a similar ratio to the third  $F_3$  group. If the  $S$  is  $AaBb$ , and the gametes are  $1AB:nAb:naB:1ab$ , we could explain such ratios. But there is nothing else to warrant the assumption of such unequal gametic segregation.

TABLE VI. *O. valdiviana*  $F_4$ .

	Longs	Mids	Shorts
$M \times S$	0	124	113
$S \times M$	0	108	89
Totals	0	232	202
$M \times S$	2	50	87
$S \times M$	3	53	44
Totals	5	103	131
$M \times S$	51	38	80
$S \times M$	84	76	133
Totals	135	114	213
$M \times S$	27	28	58
$S \times M$	8	4	10
Totals	35	32	68

In 1913 from the third group, giving  $51L:38M:80S$ , a further generation ( $F_5$ ) was raised giving  $M19/4 \times S19/1 = 0L:21M:33S$ , and the reciprocal  $S19/1 \times M19/4 = 0L:47M:53S$ , again good evidence of an  $M \times S$  ratio giving no  $L$ 's. Note the excess of Shorts.

From the first of these crosses three Mids were selfed. The first gave a total of  $10L:44M$ , or giving the capsules separately:

	Longs	Mids	
	1	9	
	0	2	
	4	9	
(26/1)	1	2	
	4	15	setting seed spontaneously under cover
	0	7	" " "
	10	44	

Many of these Mids had abnormal short and long pollen, individual stamens growing at heights not in accordance to rule. The seed raised from  $F_5$  was all lost, as it was kept until 1919 when it was found that it had lost its power of germination.

A second Mid 26/2 gave  $2L:7M:4S$ .

And a third Mid 26/3 gave  $1L:7M:1S$ .

Two of these Mids were also tested when crossed with two different Shorts.

	Longs	Mids	Shorts
$S(5/1) \times M(26/1)$ gave	0	13	12
$S(5/1) \times M(26/2)$ "	2	14	25
$S(5/2) \times M(26/1)$ "	7	12	17
$S(5/2) \times M(26/2)$ "	10	3	15

Here we get fair accordance with the belief that

$$S5/1 = AaB \text{ and } M26/1 = aBab,$$

$$S5/2 = Aab \text{ and } M26/2 = aBab.$$

But there is another piece of conflicting evidence. For  $M26/2$  selfed gave 4 Shorts, where none were anticipated, and this recurrence of Shorts in the selfed Mid families is too frequent to be accounted for by error.

I also got irrefutable evidence that  $M$ 's can differ in constitution in a manner unexplained by the scheme. Four different Mids with the same Short gave (reciprocals added together): 0:58:59, 0:34:28, 8:8:8, and 10:17:29. These same four Mids, all crossed with another Short, gave in the same order 0:57:44, 0:83:71, 127:106:205 and 25:15:39. The two first Mids must of necessity differ from the two last.

The final conclusion of this paper must be the unsatisfactory one that the only scheme so far suggested by no means fits all the facts. That Long =  $aabb$ , Mid =  $aaBb$ , and Short =  $Aabb$ —so promisingly simple at the outset—cannot possibly be the whole of the story, as observed phenomena have testified again and again. Nor will a substituted scheme, taking  $L$ ,  $M$  and  $S$  as Multiple Allelomorphs, take us any further, that we can see.

The above work was carried out at the John Innes Institution, and I should like to take this opportunity of expressing my thanks for all the facilities that have been afforded me. I should like to thank Miss Irma Andersson for her help in 1919; also the members of the Staff who have recorded families and collected seed for me when I could not be present.

Whilst this was going through the Press, a further paper has appeared by v. Ubisch (*Zts. f. Bot.* xv.), which I cannot discuss here. Our main difficulties are unaltered.

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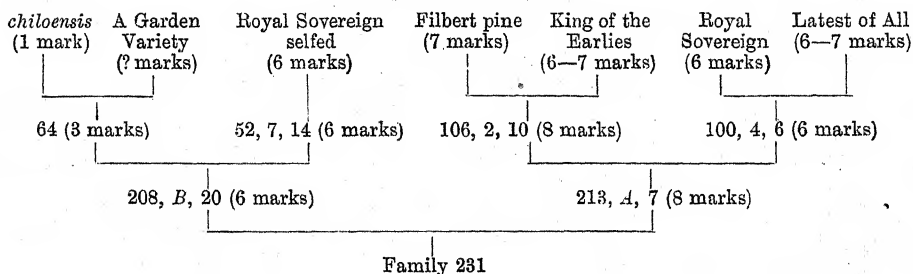
# NOTES ON *FRAGARIA*.

By C. W. RICHARDSON.

## *Flavour.*

My former system of awarding marks for flavour (*Journ. Gen.* Vol. x. p. 41) included both bad and poor plants; with their disappearance I have modified the marking, giving a wider range, so that a plant with fruit of no flavour receives but one mark and a plant, such as Royal Sovereign, with fruit of good flavour six marks.

### *Family 231. Pedigree.*

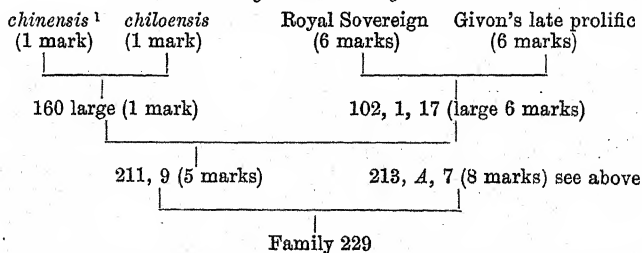


Plants were vigorous and their fruit was marked: 1 plant received 1 mark, 1—2, 3—3, 1—4, 2—5, 3—6, 4—7 and 4 received 8 marks.

This is the first family I have raised which produced no fruit of bad flavour. The degree of excellency ascends fairly steadily.

A closely related family 232 with parents (the first number denotes the family, the letter and last figure the plant) 213, A, 16 × 208, B, 20 gave similar results, but as the number of plants was small the marking was not recorded.

### *Family 229. Pedigree.*



<sup>1</sup> The use of the name *chinensis* seems to be justifiable as these are plants reputed to be Chinese in America, a reference to which I recently discovered in *The Journal of Heredity*. The plants I have, which were obtained from Kew, are a variety of the large species *chiloensis*. The *vesca* and *chiloensis* species afford a good example of the Law of Homologous Series in Variation (*Journ. Gen.* Vol. xii. No. 1), both having Alpine, hairy coloured-flowered, double-flowered, &c., &c., forms.

Plants were very vigorous and their fruit was marked: 2 plants received 1 mark, 3—2, 4—3, 2—4, 4—5, 6—6, 4—7 and 4 received 8 marks. Here again no plants produced bad fruit. The degree of excellency ascends fairly steadily but stops somewhat, about the sixth mark.

When plants are self-fertilised I have found such a large number of the descendants produce small fruit, are lacking in stamina and quality, and are frequently inclined to be sterile that it has been impossible to judge the relative values of the fruit. In all such cases the parent plants were selected for good qualities, so it would appear that self-fertilisation constitutes a bar to the production of satisfactory  $F_2$  families; to what extent the mating of brother and sister plants, or flowers, produces a similar result I have as yet insufficient data on which to express an opinion; but the results of out-crossing seem to be the more satisfactory. Till a family is produced with fruit of uniform and high excellency (say of 8 marks or more) it will be impossible to state accurately the number of factors which are concerned in flavour. Up to the present I have been unable to judge of the values of small fruit derived from such parents as Alpines and *vescas*, but there is marked segregation of flavour. A cross (*vesca*  $\times$  *virginiana*)  $\times$  *chinensis* gave a large family which included several with marked *vesca* flavour, but unfortunately a large number of the plants were sterile, or only slightly fertile, so no conclusions could be drawn; the dominance of flavour is however noteworthy as both *virginiana* and *chinensis* are without flavour in England.

#### *Foliage.*

When *vesca* and *elatior* plants are crossed with *chinensis* the resulting hybrids have different types of foliage, *vesca*  $\times$  *chinensis* resembles *chinensis*, *elatior*  $\times$  *chinensis*, *elatior*, but in each case the leaves are larger than those of either parent plant. So female-sterile are these crosses that I have never as yet been able to obtain a viable seed from them. The (*elatior*  $\times$  *chinensis*)  $F_1$  crossed back with *chinensis* gives plants with *chinensis*, *elatior* and intermediate foliage. The leaf colour, shape and substance are not linked. The crosses I have made between *vesca*  $\times$  *chinensis* and *vesca*  $\times$  *chiloensis* have produced little or no good pollen and have been female-sterile. From a cross *vesca*  $\times$  *virginiana*, producing a *virginiana* type of foliage in the  $F_1$ , I have only obtained, by self-fertilisation, some dozen plants; their foliage closely resembles that of the  $F_1$ . Crossed back with *virginiana* the  $F_1$  gave only *virginiana*-like plants; the cross-back with *vesca* has so far failed. *Chinensis*  $\times$  (*vesca*  $\times$  *virginiana*) gave a family uniformly dark green but with many different

shapes of leaf. A small  $F_2$  family from hermaphrodite plants closely resembles the  $F_1$  in colour, but some plants have thick, leathery leaves of the *chiloensis* type, whilst others resemble the cross *chiloensis*  $\times$  *virginiana*, a somewhat *virginiana* intermediate. (*Elatior*  $\times$  *chinensis*)  $\times$  *virginiana* gave plants with leaves of *virginiana* shape and type in general, but their colour was intermediate between *elatior* and *virginiana*. This family flowered this year (1922) for the first time and proved very sterile.

The work done by others in crossing *elatior* and *virginiana* (a cross I have attempted to make, without result, very many times) seems to have resulted much as *vesca*  $\times$  *virginiana* in producing descendants of one type resembling *virginiana*. In my cross the  $F_2$  family was so multifoliate that the actual leaf shape was undefined. The fact that these plants crossed with *chinensis* gave various types of foliage points to the necessity of a very close examination of the  $F_2$  plants, before it can be definitely stated that *only virginiana*-like plants are produced. On the evidence it is rash to rush to the conclusion that *virginiana*  $\times$  *elatior*, or *vesca*, produces a parthenogenetic form.

Multifoliate leaved plants, when selfed, do not breed true to the character. A plant of 208 family, with extra leaflets well down the leaf stalk, was selfed in 1920 and young, vigorous plants were placed in the open in 1921; none of these have produced multifoliate leaves up to August 1922. The same plant when crossed back with a garden variety produced a majority (considerable) of multifoliate plants. Any strong-growing cross may have multifoliate leaves, but, as the strength of their inbred descendants departs, so the character also seems to vanish, which is not the case with flower or fruit-doubling, these characters apparently following the ordinary Mendelian rules. The most degraded degenerate of a garden plant may have diminutive coxcomby fruit, but multifoliate plants are generally vigorous.

After many endeavours to arrange leaves in some order for classification I have been obliged, up to the present, to give up the idea—the difference between one leaf and another may be a matter of personal opinion, age of leaf, or time of year. It is possible to say such a leaf is of *chiloensis* type, but it is rash to say such another is of *virginiana* or *vesca* type.

#### *Sterility and Fertility.*

When distinct species are crossed only a small percentage of ovules are fertilised and the resulting plants are almost completely female-

sterile and as a rule nearly male-sterile. When "garden" varieties are crossed with pollen from such hybrid parents the resulting plants may be graded in respect of male and female sterility, and may be represented thus:

0	0	0	1	2	2	2	1	0	in maleness
♂	♂	♂	♂	♂	♂	♂	♂	♂	
0	1	2	2	2	1	0	0	0	in femaleness

in many cases hermaphrodites do not appear, and in others steriles are absent.

A mating *virginiana* ♂ × *virginiana* ♀ gave ♂♂, ♀♀ and ♀♀, a ♀ selfed gave 29 ♀♀—7 ♀♀ but some of the ♀♀, held over to the following year, produced flowers one might call  $\frac{1}{3}\phi$ , and when examined

after another year they were still  $\frac{1}{3}\phi$ .

(*Elatior* × *chinensis*) × a garden variety set about 20 % of seed, of which a small number germinated. Eleven fully grown plants flowered and, after frequent examination for two years, proved to be—3 steriles, 1 slightly female, 1 female, 1 slightly male- and female-fertile and 5 males of various degrees of fertility. The most fertile female when mated to brother pollen set 38.67 % of seed, which all germinated (the best flower out of 4). When mated with "bush Alpine" nothing set. When crossed-back with a garden variety 90.3 % set and germinated. The plants of the brother × sister mating are robust, and, after one year in the open, seem to be of normal size, but those from the cross-back are extra-strong growers of great size. None of these plants have had time to flower. The same female pollinated with *nilgirensis* set nothing. As 90 % of the ovules of a flower frequently produce seed which germinates, it is difficult to find a reason for the *partial* sterility of such a flower when crossed with another species. Still more difficult is it to explain the fertility of a female when crossed with some pollen and her sterility when crossed with other and equally good pollen.

#### *Runnerless character.*

The cross runnerless, single and white-flowering × runnered, double and pink-flowering gave in the  $F_1$  the expected runnered, single pink. The  $F_1$  selfed, gave 53 runnerless, 130 runnered. When these figures are added to those previously obtained from crossing of single flowered plants, the total stands—97 runnerless and 342 runnered. The figures suggest a 3—1 on the average, but there is great discrepancy between



the various families. This may be due to the fact that there is always a large loss in the  $F_2$  family from sickly plants. I recorded this loss in the single runnerless  $\times$  double runnered which amounted to 85, of which 33 were traceable to the failure of seed to germinate, and this with only 183 surviving plants! Another difficulty arises, as some plants produce flower trusses which change into runners, thereafter sometimes remaining, strictly speaking, runnerless, and at other times producing normal runners. As, I am inclined to think, such flowers invariably occur on very tight and tall-foliaged plants, which necessitate the flower-stems growing to a great length, it seems more than likely that the apparent change to runners is merely an excessive growth of adventitious roots. I have found such root-producing trusses in normal runner-making plants and encouraged them to root, but up to the present in vain. There are too plants producing runners which flower before they are rooted. The bud which produces the flower on the runner is comparable to that in fruit trees which produces the flower on the branch. There seems to be no rule in the  $F_2$ 's as to whether a plant shall flower before it makes runners or make runners first, in the usual way; also their time of first flowering varies from one month to over a year. In connection with the period of time between germination and flowering it is worth noting that seed sown in June flowered occasionally in October (under glass) and regularly the following summer; whereas similar seed sown in September did not flower the following summer, the plants in each case being treated in exactly the same way. *Vesca* and *alpina* seed generally flowers within six months or a year of planting; but I find *elatior* (hautbois) takes twelve to eighteen months, and some American and Asiatic light-leaved plants follow the *vesca* or hautbois example. The light-leaved strawberry is, to my mind, a very fine example of the utility of naming varieties and the futility of grading them into species, sub-species and varieties of sub-species.

To return to the original cross; the double white runnerless and double pink runnerless appeared, but I had to leave my runnerless plants before all of them had flowered (eleven months after sowing), so actual figures are not to hand and in any case would be too small to be of value. The double flowers were frequently of the "hen and chickens" type, a departure from the original white double parent. The introduction of the Alpine strain may account for the overflow of vitality in the flower, as my former Alpine  $\times$  *vesca* crosses produced very strong-growing plants.

*Fruit colour.*

Red, dark red and light red are all dominant to white. Red of the ordinary *vesca* or Alpine density is dominant to dark red (so-called black) and the lighter shades of red. Up to the present I have no facts on the cross light red and dark red. Very light red is constantly confused with white; fruit from supposed white plants should never be gathered till it drops or begins to wither, if accurate information is required as to colour.

*Variegation.*

There are, at least, two forms of variegation in the foliage of strawberries, and both fluctuate. Seed sown from the form with white splashed leaves produces yellow, green, splashed yellow and green, splashed white and green and pure white cotyledons. Seed sown from mottled green and yellow produces yellow, green, mottled yellow and green and very pale yellow, but apparently no white, cotyledons. All my pure whites and yellows died off after producing one or two leaves; the "splashed" forms also died, but as only a very few appeared, and the green splashes were small, it is more than probable that green forms with white splashes would survive. Only a small percentage of pollen from variegated plants seems good and I have failed to self any plants, making use of bags, cages and a protected greenhouse. Tested on three flowers of another variety the pollen failed with the exception of two seeds setting on one flower. Whilst one cannot say malnutrition is the cause of variegation in strawberries, plants when well nourished develop chlorophyll in sufficient quantity to cover the entire leaf; but frequently leaves from such plants are badly developed. Owing to the fluctuating character the strawberry is not a good subject for the study of variegation.

I regret to say I have been obliged to give up my long association with The John Innes Horticultural Institution, as I am about to settle in South Africa. Whilst many varieties of *Fragaria* can be grown in my new country, I expect still more will be difficult or impossible to cultivate. I fear too my work on flavour may require some further modifications, as I have reason to suppose rapidity in ripening tends to reduce flavour; that this may not invariably be the case is more than possible. Some flowers seem to lose their scent in South Africa yet others of the same species (e.g. Roses) retain their full fragrance.

# ON THE CROSSING OF SOME SPECIES OF COLUMBIDAE, AND THE INHERITANCE OF CERTAIN CHARACTERS IN THEIR HYBRID OFFSPRING.

By RICHARD STAPLES-BROWNE, M.A., M.B.E., F.Z.S.

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## I. INTRODUCTION AND BRIEF STATEMENT OF RESULTS.

The experiments herein described form part of a larger series on heredity in pigeons, of which three accounts have already been published, namely in the *P. Z. S.* for 1905, Vol. II. p. 550, the *P. Z. S.* for 1908, p. 67, and the *Journal of Genetics*, Vol. II. p. 131. The first of these papers deals with the characters of the webbed foot and "shell," the other two with colour. In the two reports on colour the experiments are numbered consecutively, Exps. 1-46 being described in the *P. Z. S.*, and Exps. 47-91 in the *Journal of Genetics*, and reference is made in the present report to birds raised in these experiments.

The crossings with which this account deals were made between wild species either mated *inter se* or with domestic pigeons, and are not numbered in series with the rest.

The experiments were brought to a conclusion by the war, and I had hoped that, before publishing an account of them, I should have been able to investigate the matter further. As this has not been possible, I think it best to give the present account of the crossings as far as they were carried.

Hybrids of the Stock Dove (*C. ænas*) and the Woodpigeon (*C. palumbus*) respectively with domestic pigeons have from time to time been obtained, and descriptions of them have appeared. A notable instance was a paper by P. St M. Podmore in the *Zoologist* for November 1903 in which he described a fertile ♂ hybrid between *C. palumbus* and a domestic pigeon, which, when mated to a domestic ♀, gave a fertile ♀ hybrid. In 1908 he presented to the British Museum a hybrid produced from the ♂ and ♀ hybrids mentioned in his paper. More recently Ghigi<sup>1</sup> has succeeded in obtaining fertile ♂ hybrids from domestic pigeons and *Columba leuconota*, which, when mated to domestic ♀s, gave offspring fertile *inter se*.

I was fortunate in having some undoubtedly pure specimens of the Rock Dove (*C. livia*) and, apart from the question of fertility, there thus appeared to be a good opportunity of testing further the character of the white rump, with which I have already dealt in my second report on the inheritance of colour. The other species used, *C. schimperi* (the Egyptian Rock Dove), *C. ænas*, and *C. palumbus*, have no white on the rump. On the other hand *C. palumbus* has a patch of white on each side of the neck, and a broad edge of white to the wing-coverts, forming a conspicuous bar, two characters which are absent in *C. livia*.

In other respects the species differ in the colour of the breast, which is a rich vinous purple in *C. palumbus*, and the same colour, though in a less degree, in *C. ænas*. In *C. livia*, however, there is hardly any tinge of vinous, the breast being grey. Again the well-marked black wing bars seen in *C. livia* are considerably reduced in *C. ænas*, and completely absent in *C. palumbus*. The species also differ in size, shape and note.

The other object I had in view was to compare the result of mating these various species to white pigeons, with the series of experiments already described in the *Journal of Genetics* on the mating of *C. livia* to white pigeons, with reference to the possibility of other colours or patterns segregating out.

The results obtained may be summarised briefly as follows:

As regards behaviour of the species, the mating of *C. schimperi* with a domestic pigeon (Exp. 1) was only carried far enough to show the readiness with which these birds breed. *C. ænas* (Exps. 2-7) also paired with *C. livia* and domestic pigeons. When mated with domestic pigeons, it produced hybrid ♂s which were fertile, but bred more readily in their second or third year. These  $F_1$  hybrid ♂s, when themselves mated to domestic pigeons, produced a weakly  $F_2$  generation, which, however, con-

<sup>1</sup> *Revista Italiana di Ornitologia*, 1919.

tained one ♂ which showed unusual sexual precocity. It was found to be difficult to get *C. palumbus* to breed either with *C. livia*, or with domestic pigeons (Exps. 8-12). Only one hybrid was obtained from these matings, this was ♀ and showed no inclination to breed even in its second year.

The shape and, to some extent, the size of *C. palumbus* was dominant, as was also the note and attitude of the male *C. ænas*, in the  $F_1$  hybrids. The vinous colour of the breast of *C. palumbus* and *C. ænas*, and the black wing bars of *C. livia* were present in the  $F_1$  hybrids, but in less degree. The patch of white feathers at the side of the neck of *C. palumbus* was never developed in the hybrid, but the broad white edge to the wing-coverts was present.

The inheritance of the white rump character of *C. livia* gave an altogether unexpected result. As has previously been shown, this character is dominant to the blue rump. The hybrid, however, produced from the mating of *C. palumbus* with *C. livia* in Exp. 9, and probably also those from the mating of *C. ænas* with *C. livia* in Exp. 2, had no white on the rump. On the other hand the mating of a white domestic pigeon to *C. ænas* in Exp. 3 gave  $F_1$  hybrids with white on the rump.

In the mating of *C. ænas* with a white domestic pigeon (Exps. 3-7) the white character was found to behave as an ordinary Mendelian recessive as before. A chequer character, in which the blue feathers of the wing-coverts and back are dappled with black, was introduced by the white pigeons used, and was again seen to behave as an ordinary dominant to the non-chequered form, but the numbers obtained in Exp. 7 differ somewhat from the expected ratio.

As many of the young hybrids died in the nest when only a few days old, their sexes were not determined, but, of those that were reared, it was noticed that the great majority were male, and, in Exp. 6, a family of seven birds was raised, of which every one was male. The fact that no female hybrid from *C. ænas* ever reached maturity, either in  $F_1$  or in the generation produced from the mating of  $F_1$  with domestic pigeons, prevented the testing of the fertility of the hybrids when mated *inter se*.

## II. WILD SPECIES USED IN EXPERIMENTS.

*C. livia* ♂ (a) and *C. livia* ♂ 26 were both bred in captivity from a pair taken at Achill Island. The former was previously used in Exp. 50<sup>1</sup>, the latter had not been mated before.

<sup>1</sup> *Journal of Genetics*, Vol. II. p. 144.

*C. schimperi* ♀ was taken from the nest in Egypt.

*C. ænas* ♂ (a) and *C. ænas* ♀ (b) were bred in captivity from a pair taken at Fen Ditton, Cambridgeshire.

*C. palumbus* ♂ (a) and *C. palumbus* ♀ (b) were taken from the nest at Fairford, Gloucestershire.

### III. DOMESTIC PIGEONS USED IN EXPERIMENTS.

White ♀ 4 was raised in Exp. 54<sup>1</sup>, being in  $F_2$  from a cross between a white Fantail ♀ and a typical *C. livia* ♂ bred from a pair obtained from Lincolnshire, the  $F_1$  generation being blue chequer with some white feathers. This ♀ was mated to *C. livia* ♂ (a) in Exp. 50<sup>2</sup> and gave two blues with white feathers, of which one, Blue w. f. ♀ 7 (*v. infra*) was used in Exp. 7 of the present series.

White ♀ 9 was raised in Exp. 8<sup>3</sup> in  $F_3$  from a cross between black Barb and white Fantail pigeons, the  $F_1$  and  $F_2$  birds in its ancestry being black with some white feathers.

White ♀ 31 and white ♂ 10 were produced in Exp. 66<sup>4</sup> from a pair of blue chequers with white feathers, derived from the original crosses of black Barbs and white Fantails, and also from Lincolnshire *C. livia* and white Fantail.

White ♂ 53 was raised in an experiment which has not yet been described. As no offspring were produced from its mating, the details of its ancestry are unimportant.

Blue w. f. ♀ 7 was produced in Exp. 50<sup>5</sup> from the mating of white ♀ 4 with *C. livia* ♂ (a). It was blue with black wing and tail bars, and had some white feathers on the rump and thighs, and also some white flights and tertiaries. No trace of chequering was seen. This ♀ was mated to its brother Blue w. f. ♂ 6, a bird of exactly similar appearance in Exp. 51<sup>6</sup> and gave 2 typical *C. livia*, 4 blue with much white, and 1 white. From the results of Exps. 50 and 51 it was presumed that white ♀ 4 was homozygous for the non-chequer character, but the result of its mating with *C. ænas* in Exp. 3 of the present series suggests that it was heterozygous for that character, and that, had Exp. 50 been prolonged, chequered birds would have appeared.

The pedigree (p. 158) of the Domestic Pigeons used shows the

<sup>1</sup> *Journal of Genetics*, Vol. II. p. 147.

<sup>2</sup> *Ibid.* p. 144.

<sup>3</sup> *P. Z. S.* 1908, p. 79.

<sup>4</sup> *Journal of Genetics*, Vol. II. p. 152.

<sup>5</sup> *Ibid.* p. 145.

<sup>6</sup> *Ibid.* p. 145.

above mentioned birds in heavy type. The following abbreviations are used:

- Wh. Fan. = White Fantail.
- Bk Barb = Black Barb.
- Bk w. f. = Black, with some white feathers.
- Blue w. f. = Blue, with some white feathers.
- B. C. = Blue, chequered with black.
- B. C. w. f. = Blue, chequered with black, and having some white feathers.

#### IV. MATINGS.

Table I shows the twelve matings of *C. schimperi*, *C. aenas* and *C. palumbus* respectively to *C. livia* and domestic pigeons, and the further matings of the hybrid offspring. The experiments are arranged for each species in the order in which they were made.

They fall naturally into two classes:

- (1) The mating of wild species together.
- (2) The mating of wild species to domestic pigeons.

The mating of wild species together is shown in Exps. 2, 9, and 12, and that of  $F_1$  hybrid to a species in Exp. 10.

The mating of wild species to domestic pigeons is shown in Exps. 1, 3, 8, and 11, and that of  $F_1$  hybrids to domestic pigeons in Exps. 4, 5, and 6, and of  $F_2$  hybrid to domestic pigeon in Exp. 7.

The table also shows the number of eggs laid by each pair, of young hatched, and those reared to maturity.

#### V. BEHAVIOUR AND FERTILITY OF THE WILD SPECIES.

##### (a) When mated together.

1. *C. aenas* ♀ (b) × *C. livia* ♂ (a). Exp. 2.

This pair was mated on February 2. One unfertile egg was laid on May 15. Subsequently a pair of eggs was laid which hatched on June 29, but the young only survived a few days. *C. livia* ♂ was extremely pugnacious with *C. aenas* ♀, and on two occasions the pair had to be separated for a few days. On July 16 the ♀ died.

2. *C. palumbus* ♀ (b) × *C. livia* ♂ (a). Exp. 9.

*C. palumbus* ♀ (b) × *C. livia* ♂ 26. Exp. 12.

*Pedigree of Domestic Pigeons used in these Crosses.*

(Birds used are in heavy type.)

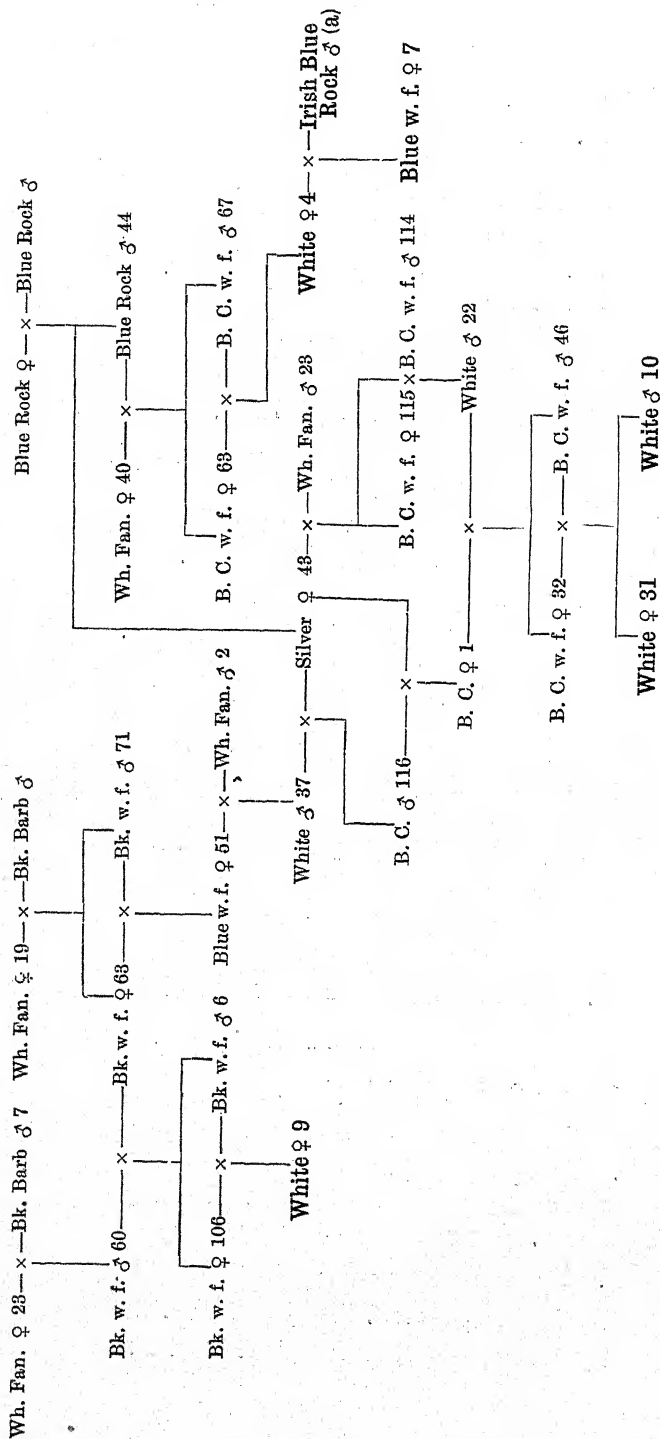




TABLE I.

Experiment Number	Females	Raised in Experiment	Also used in Experiment	Males	Raised in Experiment	Also used in Experiment	Eggs laid	Eggs fertile	Birds hatched	Colour visible	Birds reared
1	<i>C. schimperi</i> ...	—	—	White 10	Rep. II. 66	—	2	2	0	0	0
2	<i>C. canas</i> (b) ...	—	—	<i>C. livia</i> (a)	...	9	3	2	2	2	0
3	White 4	Rep. II. 54	4, 8	<i>C. canas</i> (a)	...	—	10	8	8	3	2
4	White 4	Rep. II. 54	3, 8	Hybrid 13	...	6	2	0	0	0	0
5	White 9	Rep. I. 8	—	Hybrid 37	...	3	8	2	2	2	0
6	White 31	Rep. II. 66	—	Hybrid 13	...	3	8	7	7	7	6
7	Blue w. f. 7	Rep. II. 50	—	Hybrid 1818	...	6	12	12	12	12	9
8	White 4	Rep. II. 54	3, 4	<i>C. palumbus</i> (a)	...	—	0	0	0	0	0
9	<i>C. palumbus</i> (b)	—	11, 12	<i>C. livia</i> (a)	...	2	6	1	1	1	1
10	Hybrid 60	9	—	<i>C. livia</i> 26	...	12	0	0	0	0	0
11	<i>C. palumbus</i> (b)	—	9, 12	White 53	...	—	4	0	0	0	0
12	<i>C. palumbus</i> (b)	—	9, 11	<i>C. livia</i> 26	...	10	13	0	0	0	0

Note. Rep. I = "First report on the inheritance of colour in Domestic Pigeons with special reference to reversion," *P. Z. S.* 1908, p. 67.

Rep. II = "Second report on the inheritance of colour in pigeons, together with an account of some experiments on the crossing of certain races of doves, with special reference to sex-limited inheritance," *Journal of Genetics*, Vol. II. p. 131.

TABLE II.

Experiment Number	Females	Raised in Experiment	Also used in Experiment	Males	Raised in Experiment	Also used in Experiment	Offspring			
							Blue no white	Blue some white	Blue chequer some white	White
2	<i>C. canas</i> (b) ...	—	—	<i>C. livia</i> (a)	—	9	2?	—	—	—
3	White 4	Rep. II. 54	—	<i>C. canas</i> (a)	—	—	—	—	3	—
5	White 9	Rep. I. 8	—	Hybrid 37	3	—	—	—	—	2
6	White 31	Rep. II. 66	—	Hybrid 13	3	—	—	—	4	3
7	Blue w. f. 7	Rep. II. 50	—	Hybrid 1818	6	—	1	5	1	4
9	<i>C. palumbus</i> (b)	—	—	<i>C. livia</i> (a)	—	2	—	1	—	—

Note.. The above table includes only those matings from which young birds were hatched.

The ♂ used in Exp. 9 is the one which had previously produced young with *C. ænas* ♀ in Exp. 2. The birds were mated on March 30, the first pair of eggs was laid about June 10, and the second on July 2. Both pairs of eggs were unfertile. Of the third pair of eggs, one proved unfertile, and the other hatched on August 18, the hybrid (♀ 60) being reared to maturity.

During the next two years *C. palumbus* ♀ (b) was mated to a white domestic ♂. (Exp. 11, *v. infra*.)

In the fourth year this ♀ was mated to *C. livia* ♂ 20 (Exp. 12). Thirteen eggs were laid, but none of them was fertile.

(b) *When mated to domestic pigeons.*

1. *C. schimperi* ♀ × white ♂ 10. Exp. 1.

This pair was mated on March 6, and eggs were laid about March 26. On April 13 the ♀ was found dead on the nest. The eggs were removed to foster parents, but failed to hatch, a dead bird being found in each.

2. White ♀ 4 × *C. ænas* ♂ (a). Exp. 3.

Mated on February 2, ten eggs were produced by these birds, of which eight were fertile. One egg of each of the first two pairs, laid respectively on March 14 and April 18, was unfertile. Only two of the young reached maturity (hybrid ♂ 13 and hybrid ♂ 37) and these were used subsequently in later experiments. The fifth pair of eggs was laid on July 23, and *C. ænas* ♂ died on July 31.

3. White ♀ 4 × *C. palumbus* ♂ (a). Exp. 8.

The white ♀ used in this experiment was that which had produced young with *C. ænas* ♂ in Exp. 3 the year before. It was mated to *C. palumbus* ♂ on March 27, and the pair remained together till August 2. During the whole of this period the ♂ was never observed to take any notice of the ♀, and no eggs were laid.

4. *C. palumbus* ♀ (b) × White ♂ 53. Exp. 11.

*C. palumbus* ♀ (b) had previously produced a hybrid with *C. livia* ♂ (a) in Exp. 9 (*v. supra*). It was mated for two years to White ♂ 53. During the first year no eggs were laid, and during the second, four unfertile ones.

This ♀ was kept for four years, during which time it was mated to three ♂s, and produced in all 23 eggs, of which only one proved fertile.

## VI. BEHAVIOUR AND FERTILITY OF THE HYBRIDS.

(a) *From the mating together of the wild species.*

*Hybrid* ♀ 60 from *C. palumbus* ♀ (b) × *C. livia* ♂ (a).

This bird, raised in Exp. 9, showed no inclination to mate. When two years old it was put up with *C. livia* ♂ 26 (Exp. 10). The ♂ apparently took no notice of the hybrid, and no eggs were laid. On dissection the ovary was found fully developed.

(b) *From the mating of wild species with domestic pigeons.**F<sub>1</sub> Generation.*

*Hybrid* ♂ 13 and *Hybrid* ♂ 37.

These two birds were raised in Exp. 3 from White ♀ 4 × *C. œnas* ♂ (a), and were reared to maturity. Only one (No. 13) showed any inclination to pair the following summer. It was therefore mated on August 2 to its own mother, White ♀ 4 (Exp. 4) and two eggs were laid about the 16th. These were unfertile. The following year it showed no inclination to breed, but, in its third year, it was mated to White ♀ 31 (Exp. 6). Eight eggs were laid, of which only one proved unfertile. Of the seven young produced, all but one reached maturity, but only hybrid ♂ 1818 survived during the next summer.

Hybrid ♂ 37 showed no desire to breed until two years old. It was then mated to White ♀ 9 (Exp. 5). Four pairs of eggs resulted, the first two pairs being unfertile. One egg of the third and one of the fourth pair were fertile, and two birds were hatched, but that from the third pair only survived a week. The bird raised from the fourth pair of eggs lived four months. Hybrid ♂ 37 died a fortnight after the last young bird was hatched.

*F<sub>2</sub> Generation.*

In striking contrast to the behaviour of the *F<sub>1</sub>* ♂ hybrids was that of *F<sub>2</sub>* hybrid ♂ 1818, produced in Exp. 6, which, although hatched as late as August 1, was, nevertheless, seen to make an attempt to tread a ♀ during the same winter. It was put up in the next spring with another hybrid which was believed at the time to be ♀, but subsequently proved not to be so. On August 1 it was mated to a domestic pigeon, Blue w. f. ♀ 7 (Exp. 7), and a pair of young was hatched on September 8. After the breeding season the pair was separated and remated the following January, when they produced five pairs of fertile eggs, the first pair hatching on

February 26, and the last on August 11. No unfertile egg was laid, and nine of the twelve young were raised to maturity.

#### VII. DESCRIPTION OF THE HYBRIDS.

##### (a) *From the mating together of wild species.*

###### *F<sub>1</sub> Generation.*

*C. ænas* ♀ (b) × *C. livia* ♂ (a). Exp. 2.

The two hybrids for this cross only survived a few days. The quills were dark and suggested that the plumage would have been blue *without the white rump*. Unfortunately the weather was hot at the time of their death and, when the specimens reached the taxidermist, they were found to be unfit for preservation, and so were lost.

*C. palumbus* ♀ (b) × *C. livia* ♂ (a). Hybrid ♀ 60. Exp. 9.

In general appearance this bird resembled *C. palumbus* more closely than *C. livia*. The length was 16 inches; that of the mother being 17 inches. The length of the neck and characteristic carriage of the head of *C. palumbus* was noticeable in the hybrid. The plumage also resembled that of *C. palumbus* except that the mantle, breast, and under parts were of a bluer tinge, and the vinous colour of the breast was not so marked. The conspicuous patch of white feathers, seen on the neck of the adult *C. palumbus* after the first moult, was never assumed by the hybrid, but, on the other hand, the broad white edge of the wing-coverts of the mother was equally developed in the offspring. On other parts of the plumage white feathers were entirely absent. *The rump was slate-grey*. The two black wing bars, present in *C. livia* but absent in *C. palumbus*, were seen in the hybrid, but the black was not nearly so pronounced or extensive as in *C. livia*. The upper bar was present only on the inner part of the wing. The tail feathers were, as in *C. palumbus*, nearly black, except at their bases.

##### (b) *From the mating of wild species with domestic pigeons.*

###### *F<sub>1</sub> Generation.*

White ♀ 4 × *C. ænas* ♂ (a). Exp. 3.

The eight birds hatched from this experiment had dark down in the nest, and would therefore have had coloured plumage. Only three feathered. The remaining five died very soon after hatching. The general colour of those which were raised was blue heavily chequered with black on the wing-coverts and back, and having *some white feathers which were especially noticeable on the rump*. The vinous colour of the breast

of *C. aenas* could be traced in the hybrids but was not pronounced. The black wing bars were present, as also the tail bar. In the case of hybrids Nos. 13 and 37, the tail bar was double, this feature being most marked on the more external tail feathers. The blue colour between the terminal and subterminal bars was identical with that of the basal part of the tail feathers. Hybrid No. 52 died before the tail was fully developed and consequently this feature was not visible. A slight bronzing or rustiness of some of the black colour was seen, and this did not entirely disappear at the first or any subsequent moult, as is frequently the case in domestic pigeons when it occurs in the next plumage.

The white rump was a most conspicuous feature in all three hybrids. The boundaries of the white patch were not so clearly defined as in *C. livia*, the edges being irregular, and some coloured feathers were present in the midst of the white. The amount of white on the rump varied in the three hybrids. In hybrid ♂ 13 the extent was about the same as in *C. livia*. In the other two it was less. *The amount of white on other parts of the plumage was directly proportional to the amount of white on the rump.* Hybrid ♂ 13 had a few white feathers on the head and neck, at the angle of the wings and on the thighs. Hybrid ♂ 37 had two white feathers on the head and very few on the thighs. Hybrid 52, with least white on the rump, had none elsewhere.

The plumage of these birds was softer than that of *C. livia* or the domestic pigeons, so that they, like *C. aenas*, could easily be identified by touch.

In the case of the two hybrid ♂s which were raised to maturity it was observed that the note and mating attitude were identical with those of *C. aenas*.

#### *F<sub>2</sub> Generation.*

White ♀ 9 × *F<sub>1</sub>* Hybrid ♂ 37. Exp. 5.

White ♀ 31 × *F<sub>1</sub>* Hybrid ♂ 13. Exp. 6.

As will be seen in Table II, two young birds were produced in Exp. 5 both of which were white. Of the seven raised in Exp. 6, three were white and four were blue chequers with some white feathers, making a total of four blue chequers to five whites for the two matings. The whites resembled their ♀ parent in all respects. The coloured birds varied in the depth of chequering, one being more lightly marked than the others. There was no duplication of the tail bar.

In no case did the amount of white on the rump approach that in *C. livia* or in the *F<sub>1</sub>* generation of hybrids, but, as in Exp. 3, the amount

of white on other parts of the plumage varied directly with that on the rump. The details of the distribution of white feathers were as follows :

*F<sub>2</sub> hybrid* 1808. None on rump, very few on thighs.

„      1818. 8 on rump, 2 flights, 1 tertiary.

„      1828. About 15 on rump, 3 flights and a few on abdomen, thighs, and at angle of wing.

*F<sub>2</sub> hybrid* 1292 was a deformed and very weakly bird. The left wing was rudimentary and the left foot consisted of two digits only. It died when less than two months old, and the body, when found, had been trampled on by other pigeons, so that the details of white markings were not clear.

#### *F<sub>3</sub> Generation.*

*Blue with some white feathers* ♀ 7 × *F<sub>2</sub> Hybrid* ♂ 1818. Exp. 7.

Twelve young were obtained from this mating, of which two were blue chequers, six blue with black wing and tail bars, and four white. The plumage of the blue chequers resembled that of the birds raised in the preceding experiments. The blue birds were not uniform in colouring, some being of a darker shade than others, and one (*F<sub>3</sub> hybrid* 2013) being conspicuously dark and smoky in plumage.

As regards white feathers, two showed none either on the rump or elsewhere, five showed some white feathers, and one (*F<sub>3</sub> hybrid* 5720) had a considerable amount of white. The details of the distribution of white feathers, when present, were as follows :

*F<sub>3</sub> hybrid* 2013. None on rump, 1 on head.

„      2014. Few on rump, 8 primaries, 5 tertiaries and few on head and thighs.

„      5713. None on rump, 2 primaries.

„      5716. 2 on rump, few on thighs.

„      5717. Few on rump, streak behind each eye, 7 primaries, 6 tertiaries, and few on thighs.

„      5720. Rump white. Head and throat conspicuously white with a few coloured feathers. Ten flight feathers in the right wing and eight in the left, together with the tertiaries over them white. Large patch of white on the abdomen and thighs.

#### VIII. INHERITANCE OF WHITE AND CHEQUERING.

Table II shows the numbers of coloured and white birds obtained from the mating of *C. œnas* with a white pigeon, as described above. It will be observed that the inheritance is according to the usual Men-

delian expectation. In  $F_1$  a uniform generation of coloured birds was produced (Exp. 3), and these hybrids, mated to whites (Exps. 5 and 6), gave approximate equality of coloured and white. In Exp. 7  $F_2$  hybrid mated to blue containing white, gave a result of practically 3 coloured : 1 white.

The chequer character appeared first in Exp. 3 and was presumably introduced by white ♀ 4. Although, as shown in Exp. 50<sup>1</sup>, this bird was not homozygous for the character, still the three  $F_1$  hybrids obtained were all chequered. The mating of  $F_1$  hybrid ♂ 13 to white ♀ 31 in Exp. 6 produced no coloured birds without chequering, so we may presume this ♀ also was at least heterozygous in chequering. The mating of  $F_2$  chequered hybrid ♂ 1818 to a blue ♀ containing white in Exp. 7, gave 2 chequers, 6 blues, and 4 whites. Here the number of chequers is low and that of blues high, as the three types would be expected to appear from this mating in the proportion of 3 chequers : 3 blues : 2 whites. Had the mating been continued it is probable that the figures would have approximated more closely to that ratio.

#### IX. INHERITANCE OF THE WHITE RUMP.

I have previously shown that, in breeding *C. livia*, the typical white-rumped form is dominant to the whole-coloured blue. In the present series of experiments, when *C. livia* is hybridised with *C. palumbus* (Exp. 9) and probably also with *C. ænas* (Exp. 2) *the reverse is the case*. On the other hand white ♀ 4 mated to *C. ænas* ♂ in Exp. 3, gave young with white feathers on the rump.

We may presume that white ♀ 4 was at least heterozygous for the white-rump character, and its ancestry shows this to be possible, it being produced in  $F_2$  from a cross between a typical *C. livia* and a white Fantail, but in the later matings of this series (Exps. 6 and 7) no bird was produced showing the white rump of *C. livia*, the white feathers being distributed as already described. In Exp. 7, however, two coloured birds were produced showing no white feathers.

In the case of whole-coloured pigeons (black Barbs) mated to whites, the  $F_1$  generation obtained was black with some white feathers on the rump and elsewhere (see *P. Z. S.* 1908, p. 77, Table I), these giving in  $F_2$  whole-coloured birds, coloured birds with white feathers, and whites. The fact that coloured birds carrying white generally show a certain

<sup>1</sup> *Journal of Genetics*, Vol. II. pp. 144 sq.

amount of white in their plumage, more especially on the rump, tends to obscure the inheritance of the rump character.

#### X. THE SEX-RATIO OF THE HYBRIDS.

It is a matter for regret that the sex of every hybrid was not ascertained by dissection. This, however, was not done, as, in the earlier experiments many of the young hybrids died soon after hatching, in my absence, and were not preserved. Also, when the birds from the last experiment were killed, I was unable to be present and so no dissections were made.

Of the 31 hybrids from the cross with *C. œnas*, the sexes of 14 only were determined. These however show a very great preponderance of males, the total numbers being twelve males to two females. The two birds reared in the  $F_1$  generation (Exp. 3) were both male, and when they were mated to domestic pigeons, in Exps. 5 and 6, only male offspring were reared, thus giving no opportunity for testing the fertility of the hybrids when mated *inter se*. In Exp. 5 one bird was hatched which proved to be female, but it did not survive long enough to be mated. In Exp. 6 the seven birds hatched were male, thus giving for that generation seven males, one female and one undetermined. The  $F_2$  hybrid mated to a domestic pigeon in Exp. 7 gave a family of twelve, but the sexes of only four of these were ascertained, of which three proved to be male and one female.

In the cross with *C. palumbus* the only hybrid hatched was female, and showed no inclination to mate.

Doncaster<sup>1</sup> has pointed out the frequency with which excess of males is produced in hybrid offspring, and gives instances of this in pheasants and ducks among birds<sup>2</sup>. The results here obtained suggest a similar deviation from equality in the case of hybrid pigeons.

#### XI. CONCLUSION.

I am indebted to Sir Reginald Oakes Bait for the specimen of *C. schimperi*, and to the late Mr J. L. Bonhote for those of *C. livia* and *C. œnas* used in these experiments.

<sup>1</sup> *The determination of Sex*, 1914, p. 86.

<sup>2</sup> Cf. also Haldane, J. B. S. *Journal of Genetics*, Vol. XII. Pt 2.



# ON THE GENETIC ANALYSIS OF A HETEROZY- GOTIC PLANT OF *SCOLOPENDRIUM VULGARE*.

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(With Plates IV, V.)

THERE is as yet little, if any, accurate knowledge regarding the course of inheritance of characters in the lower plants, almost all critical work having so far been done on the Flowering Plants that produce seeds. The study of genetics is, however, possible in the Ferns<sup>1</sup> and not only affords scope for testing conclusions drawn from seed-bearing plants but presents problems peculiar to plants in which the sexual generation is physiologically independent. The methods to be employed will differ somewhat from those followed in studying flowering plants in accordance with the difference in the life-history. The desirability of extending the study of plant genetics to lower plants seems to justify the account of an investigation, which (though the results are apparent in their main lines) is still in progress.

The study of the plant of *Scolopendrium vulgare* to which these notes relate was begun some six years ago but has not been systematically pursued throughout this period. In 1916, at my suggestion, Miss M. Edmonds made sowings from a large plant of the Common Hartstongue Fern which had been growing in the Moss-House of the Manchester University Experimental Grounds for a number of years. The object proposed was to ascertain if deviations from the normal type of *Scolopendrium* would appear when a large number of plants were raised. There was no suspicion in using this plant, which was at hand, that it was other than normal. It is believed to be one of a number collected near Skibbereen, Co. Cork, when the Moss House was being stocked; but its origin and past history for the purposes of scientific study must be regarded as unknown. It was a robust, entire-leaved

<sup>1</sup> Cf. the paper by Miss I. Andersson (*Journal of Genetics*, XIII, p. 1) published while this paper was in the press and referred to further below.

plant of *Scolopendrium vulgare* with an occasional indication of division at the extreme tip of a frond, as is often noticed in plants growing wild. A normal progeny, with at most the chance of detecting an occasional variant or mutation, was anticipated.

Several pots were sown with the spores and the surface of the soil became covered with the numerous prothalli; but as the young plants borne on these grew up it was evident that they included a considerable proportion unlike the normal type of the original plant. The majority of the plants resembled this in having entire leaves; the others had the leaves narrower and more or less cleft or incised at the margin presenting a very different appearance which was retained as they grew up. Owing to the departure of Miss Edmonds from Manchester the work was suspended at this stage; the original plant and a few of its descendants both of the entire-leaved and the incised-leaved types being preserved as pot plants.

I am indebted to Miss Edmonds for carrying out these preliminary cultures and for handing over to me her notes relating to the few plants that were grown on. I wish further to express my thanks to Mr E. Ashby for the care he has taken of the experiments since the work was resumed in 1919.

Before entering on the results of these more systematic cultures a brief description may be given of the original plant and of the two types that were raised from it. The differential characters are given by the form of the leaves.

The original plant had, as already stated, the appearance of a strongly grown individual of the species. It had grown for a number of years sending up crowns of leaves that always retained the normal shape and outline, except for occasional slight division at the extreme tip, and were abundantly fertile. The large plant died in the winter of 1919-1920 but a plant grown from a branch or bud borne on it was saved. The two leaves from this, represented in Fig. 1, give a fair idea of the characteristics of the plant under investigation, though they were not of the size attained when the large plant was in full vigour.

Another leaf represented in Fig. 2*a* shows a tendency to branching or sub-division at the extreme tip; this property had, as already mentioned, been noticed occasionally, but it was infrequent and never disturbed the normal appearance of the plant as a whole. The leaf represented in Fig. 2*b* was removed from the plant at the same time and is of considerable interest. As the photograph shows the two halves of the leaf are unlike. The right-hand half in the figure agrees with

the leaves of normal appearance characteristic of this plant, the lamina is broad, the margin entire and the veins do not anastomose. In the left half the lamina is narrower with indications of incisions marking off lobes, and, in relation to this, the venation was irregular, with anastomoses. The two halves of this leaf in fact agree respectively with the entire-leaved and incised-leaved individuals into which the progeny derived from spores of this plant will be shown below to segregate. It is natural, therefore, to interpret this solitary leaf as an example of somatic segregation of these characters.

Many of the plants produced on prothalli grown from the spores were like the parent. This agreement is shown by the two leaves in Fig. 3, one of which further shows the tendency to division at the tip which was as infrequent in the fully grown descendants as in the original plant.

The incised-leaved descendants when full grown contrasted with this normal form of leaf in the way shown in Fig. 4. The leaf-blade as a whole was distinctly narrower and its margin was characteristically, and more or less deeply, incised or lobed, besides being distinctly crenate with small rounded teeth corresponding to the ends of the veins. The double sori, as has been described for forms of this type<sup>1</sup>, tend to fall between the lobes so that the single sori of the pair continue along the opposed sides of the incision. All the incised-leaved plants agreed in these characters and presented as a result a common appearance contrasting with the entire-leaved plants. The leaves exhibited some differences in outline, however, some continuing into a tapering lobed tip and others having a widened, rounded, lobed termination. While this gave some appearance of variety to the incised-leaved plants, little if any significance can be attached to the differences; for the different types of leaf may occur on the same plant (Fig. 4). In the case of the leaves with a widened, rounded but incised summit, the veins and sori diverge from a point behind this that corresponds to the arrested end of the frond. This sometimes continues into a pointed tip or horn which may project on the lower side or on the upper side of the frond. These, and the incised margin, are characters met with in varieties of *Scolopendrium* to which descriptive names are applied.

In 1919 sowings were made from spores of the original plant; of two of the incised-leaved descendants of the first culture; and of two of the entire-leaved descendants of the first culture. The spores were

<sup>1</sup> Bower, "Studies in the Phylogeny of the Filicales. IV." *Ann. Bot.* Vol. xxviii. p. 410.

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sown, with due precautions to ensure purity, fairly closely on the soil, and when the prothalli had developed the cultures were watered from above and fertilisation allowed to take place without any isolation or control. By February 1920 there were numerous young plants but they were too young and small to allow of any decision as to the results of the experiment. Preliminary estimates, which were confirmed later, could be made by August when the plants were about a year old. The results to be given below were obtained by pricking out the young plants in the autumn of 1920; by July 1921 they were well grown and beginning to bear sporangia.

The culture of spores from the original plant confirmed the earlier experiment. Many of the resulting plants were entire-leaved but there was an evident proportion of plants with incised leaves in all the pots. Examples of these at the time of counting are represented in Figs. 5 and 6: 201 plants were thus obtained of which 147 were entire-leaved while 54 had incised leaves. This is a proportion of 73% entire-leaved to 27% incised-leaved, which is highly suggestive of a simple Mendelian segregation having taken place. It will be found to be confirmed by other cultures and will be discussed later.

It should be added that about one half of the plants, both entire-leaved and incised, in this experiment showed more or less forking of some of their leaves<sup>1</sup>. Since this character, which was noted in the parent, had not segregated and since it always tended to be outgrown and not manifested in the adult plants, it is left on one side for the present.

Both the incised-leaved plants from the first culture bred perfectly true, all the plants in the cultures being incised-leaved. The fact that the incised-leaved character came true was evident at a glance in these cultures, the numbers of plants grown from them and counted were 101 and 96. Forked or partly divided leaves were found in a large proportion of the young plants which however grew out of this as they became full sized.

There was a minor difference in the behaviour of the cultures of the two incised-leaved plants. In the one case the leaves were narrow and incised throughout and the character was perceptible in quite young plants (Fig. 7). In the other the leaves of the young plants often appeared as if almost normal and only gradually assumed the uniformly incised character (Fig. 8); the adult leaves of this plant are shown in Fig. 4 and are typically incised.

<sup>1</sup> The actual number of plants of this character was 69 of the 147 entire-leaved and 28 of the 54 incised-leaved. No importance can be attached to these numbers however.

The two entire-leaved plants derived from the original culture also behaved alike on their spores being sown; they both proved to resemble the original plant in their genetic constitution. From the culture of one plant of them 76 plants were raised for counting; of these 55 ( $=72\%$ ) were entire-leaved and 21 ( $=28\%$ ) incised-leaved. Some plants of this culture had divided leaves but the number was not recorded. From the other plant a larger crop was raised: of the 255 plants, 193 ( $=76\%$ ) were entire-leaved and 62 ( $=24\%$ ) incised-leaved. Indications of branching of the fronds were noticed in 104 of the entire-leaved and in 16 of the incised-leaved plants.

When these cultures are considered a very clear result is apparent as regards the characters expressed by the terms entire-leaved and incised-leaved. In the culture of closely sown spores of the original plant these two types appeared in a proportion closely approximating to 3:1. The incised-leaved descendants that have been tested breed true. The two entire-leaved descendants of the original plant that have been tested agreed with the latter in yielding entire-leaved and incised-leaved plants in the ratio 3:1. These results suggest strongly, though they do not conclusively prove, that the original plant is to be regarded as of heterozygotic nature, segregating on spore formation as regards the characters entire-leaved and incised-leaved according to the simple Mendelian ratio. How the origin of this nature of the original plant came about must remain unknown. Its genetic behaviour like a hybrid of the  $F_1$  generation is, however, clear. The incised-leaved progeny breed true like extracted recessives. The entire-leaved descendants so far tested have proved to possess the same hybrid nature as the parent, the entire-leaved character being dominant over the incised-leaved.

The missing case, so far as these cultures go, is that of the pure dominant form with entire leaves. Plants of this kind cannot be distinguished by mere inspection from the plants of hybrid nature. They would, presumably, be discovered if a sufficient number of individuals of the entire-leaved type were bred. If, however, the course of the life-history of the fern is considered, a more direct way becomes evident; and since this is of wide application to genetic studies in the lower plants it is worth while to make it clear for this particular case.

The fern plant which is composed of diploid cells forms spores; from a diploid spore-mother-cell in which the reduction division occurs a tetrad of four haploid spores is produced. Unlike the state of affairs in the flowering plant the spores are all of one kind and are shed freely from the plant. Any one spore can give rise to male and female sexual

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cells on the haploid prothallus into which it develops; it can thus produce diploid plants sexually without needing to be associated with the product of another spore.

It may be assumed that segregation of characters would normally take place at spore formation and that in the case of two allelomorphic characters these would be carried by the two pairs of spores of the tetrad respectively. Applied to the plant under consideration, this would mean that one half the spores would carry the entire-leaved and one half the incised-leaved characters; and these characters would be carried by all the sexual cells (eggs and spermatozoids) produced by the prothallial growth, however extensive, derived from a spore of the one or other kind.

In the closely sown cultures 50 % of the prothalli would thus carry the one character and 50 % the other. The chance mating on which the appearance of progeny in the Mendelian ratio depends is of the eggs and spermatozoids. It might have been expected that self-fertilisation would be so preponderant in a culture of prothalli that this ratio would not appear. The fact that in the crowded prothallial cultures from spores of this plant the sporophytes are produced in numbers which agree closely with the simple Mendelian ratio implies that mating among the sexual cells is governed by chance. The extracted recessives, representing 25 % of the chances, have been recognised; it is at present an assumption, though a highly probable one, that the 75 % of entire-leaved plants include 25 % pure dominant as well as the heterozygotic or hybrid type with the entire-leaved character dominant that has been recognised by breeding.

If instead of allowing the prothalli to grow together in a mixed culture they are isolated and cultivated singly in small pots, the diploid fern plants borne on each of the prothalli should be pure as regards the character carried by the single spore which gave rise to the latter. Since such isolated prothalli enlarge and multiply by branching, a considerable number of sporophytes of the same genetic constitution are often obtained from one spore. It is unnecessary to enlarge on the powers this simple technique gives in studying plants like the homosporous ferns, but it is worth while stating it explicitly.

The first steps in applying this method to the fern that is the subject of the investigation have been made. A number of prothalli developed from spores of the original plant have been isolated in small pots. The prothalli increased in size and branched. Fern plants have been produced on these prothalli in some cases more than 20 having been raised from

the prothallus derived from a single spore. All the plants borne on one prothallus are of the same kind but some of the isolated prothalli bear entire-leaved plants (Fig. 9) and others incised-leaved (Fig. 10). The numbers yet obtained are insufficient for determining the numerical proportion of the two types of spores, the anticipation is that they would be equal. If the interpretation of the original plant as of the nature of a hybrid segregating the entire-leaved and incised-leaved characters is correct, not only all the incised-leaved plants obtained from isolated prothalli, but all the entire-leaved also should breed true. The young plants are only commencing to bear fertile leaves so that by this method it will be more than a year before the answer to this question can be obtained.

The further description of the morphological and anatomical characters of the segregating types of plant in this fern, and (though this is hardly likely to be found) of any recognisable differences in the prothalli may be deferred until the pure, entire-leaved plants have been obtained.

The question just mentioned of the relation between characters of the haploid gametophyte and those of the diploid sporophyte, is evidently of considerable interest. It has already been raised by Professor Bateson for a variegated *Adiantum Capillus-Veneris*, in a rather special form since the character concerns the plastids; the first full account of the work on this fern by Miss I. Andersson<sup>1</sup> was published in the last number of this Journal.

The methods of genetic analysis which are thus being worked out for Ferns in which the haploid generation though independent is relatively small and simple will apply to such plants as the Liverworts and Mosses. In the case of these plants, however, the specific characteristics are largely given by the sexual generation and the inheritance of the characters of this bears directly on the origin of specific forms in the group. Every combination of characters carried by a spore is, presumably, purely extracted since the haploid generation on theoretical grounds cannot be heterozygotic. Since the powers of vegetative growth and extension of the sexual generation from a single spore is very great in the Bryophyta, the possibility that occasional hybridisation may have

<sup>1</sup> Irma Andersson, "The Genetics of Variegation in a Fern," *Journal of Genetics*, Vol. XIII, pp. 1-11. The references to earlier statements by Bateson are given in this paper, which also deals with the special methods employed in studying a fern. The particular case of the inheritance of variegation in *Adiantum* has such peculiar features that no purpose would be served by a detailed comparison with the apparently straightforward case of segregation described here.

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played an important part in the origin of new combinations of characters is very apparent for these plants. Up to the present, however, progeny appears never to have been raised from the spores of any of the hybrid Mosses that have been described. The same can, however, be said of the hybrid Ferns but the case of Mendelian segregation here described and that under investigation at the John Innes Horticultural Institution indicate that this group provides a practicable field for the extension of genetic studies to the lower plants.

### SUMMARY.

1. A normal looking plant of *Scolopendrium vulgare* of unknown origin proved, when its spores were sown, to be heterozygotic, and to behave like a hybrid plant of the  $F_1$  generation.

2. The progeny when the spores were sown together showed segregation of the entire-leaved and incised-leaved characters. There were about 75% of the former and 25% of the latter type.

3. The incised-leaved plants bred true, behaving like extracted recessives.

4. The two entire-leaved plants of the  $F_2$  generation that have been bred from have segregated in the same way as the original plant. They behave as impure dominants; the entire-leaved character being completely dominant to the incised-leaved.

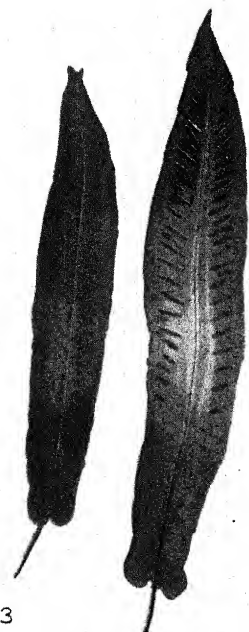
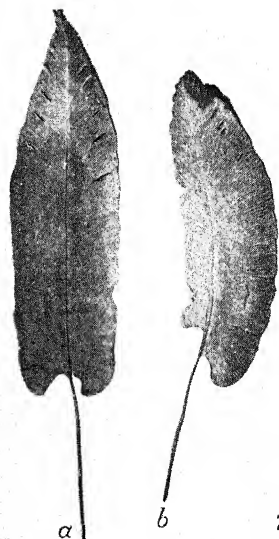
5. Pure dominant plants have not so far been proved to be present in the  $F_2$  generation, though there is no reason to suspect their absence.

6. Isolated single prothalli produce either entire-leaved or incised-leaved plants, not both. Assuming that segregation occurred at spore formation, plants thus obtained should be genetically pure. This is the most direct method of obtaining all possible pure combinations in a homosporous fern. It also applies to the genetic study of the Bryophyta.

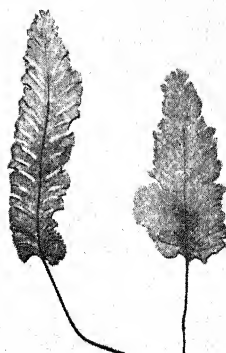
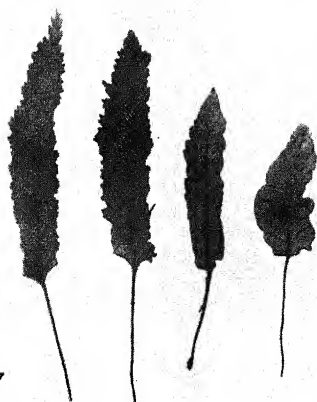
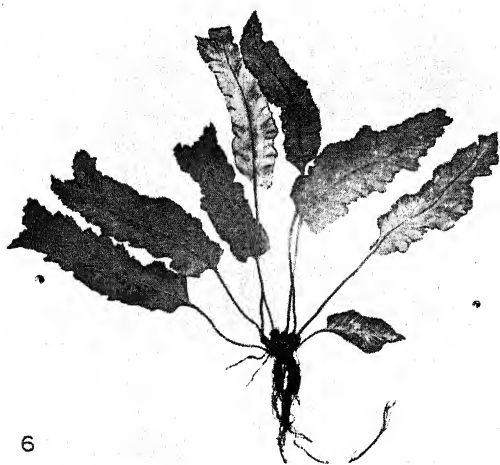
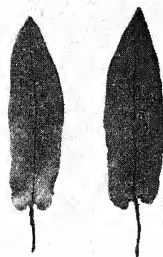
7. The original plant showed somatic segregation of the entire-leaved and incised-leaved characters in the two halves of one leaf.

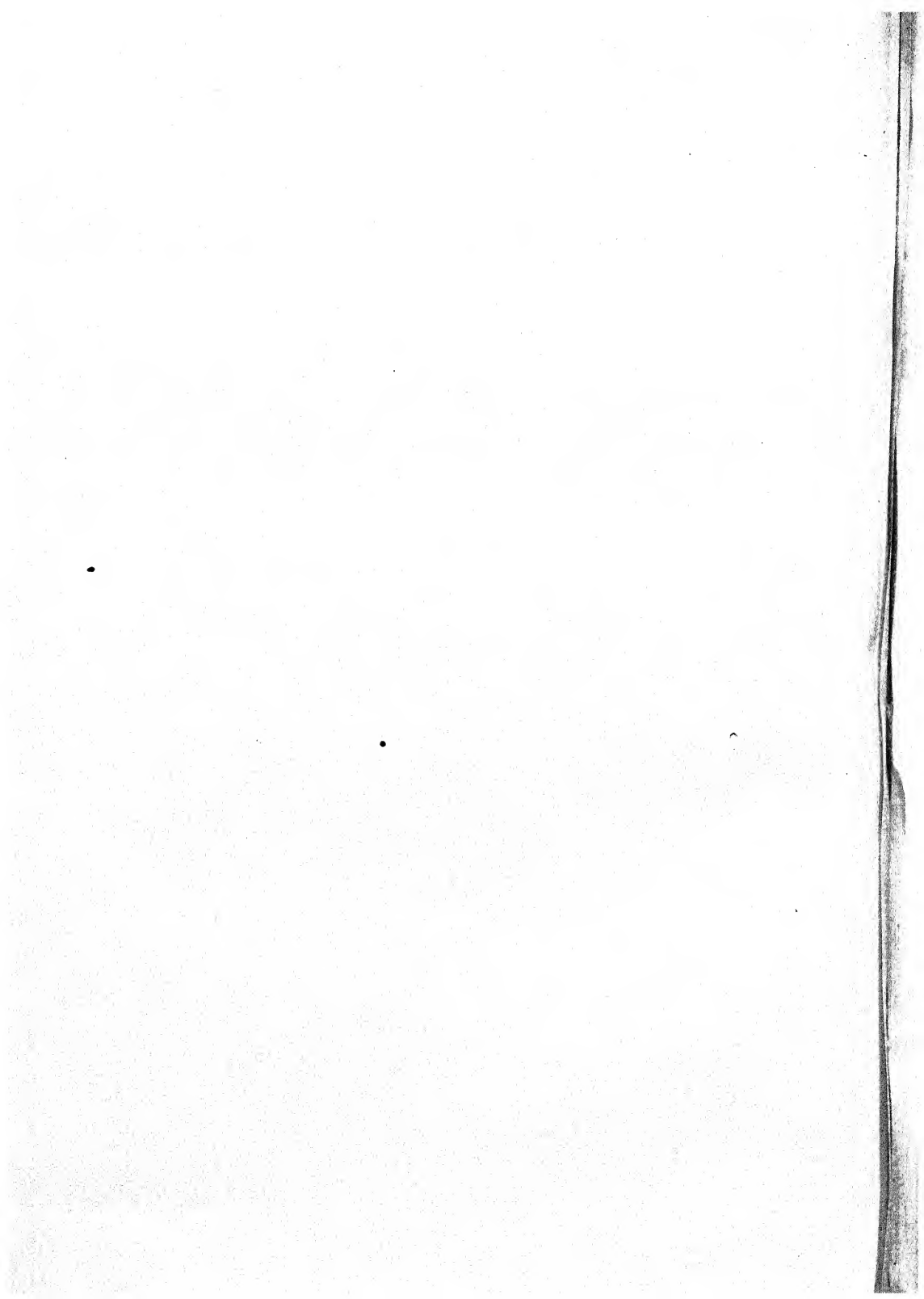
BARKER CRYPTOGAMIC RESEARCH LABORATORY,  
UNIVERSITY OF MANCHESTER.











## DESCRIPTION OF FIGURES.

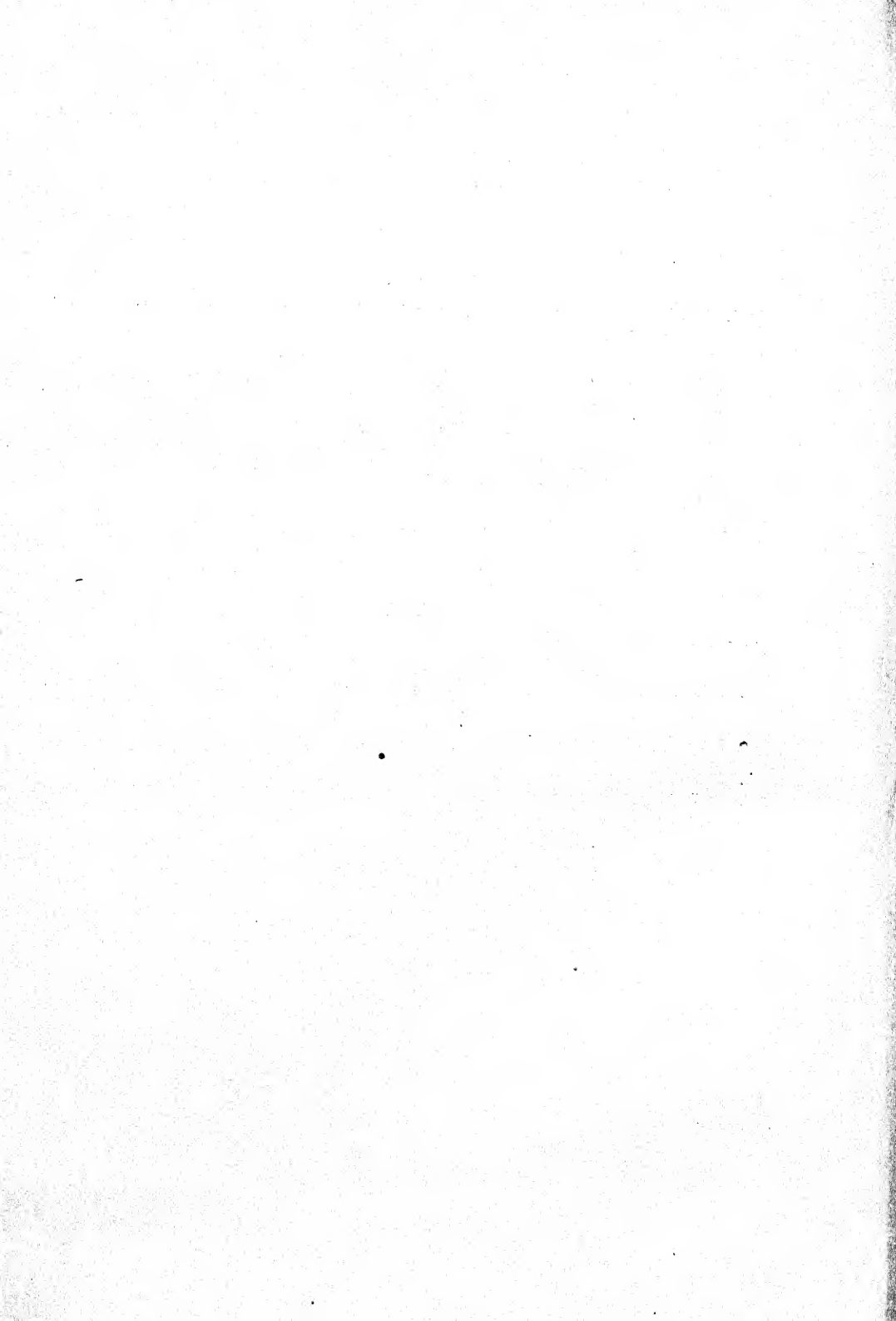
All reduced  $3\frac{1}{2}$  times.

## PLATE IV.

- Fig. 1. Two leaves of the original plant under investigation corresponding to a plant of the  $F_1$  generation.
- Fig. 2. Two small leaves of a bud or branch of the original plant.
- (a) shows the normal entire form and also an indication of sub-division at the tip.
- (b) shows somatic segregation of the incised-leaved character (to the left) the right half preserving the normal entire character.
- Fig. 3. The leaves of a plant (50) of the  $F_2$  generation showing the entire-leaved character of the impure dominants. The leaf on the left has an indication of sub-division at the tip.
- Fig. 4. Three leaves of a plant (26) of the  $F_2$  generation showing the incised-leaved character of the extracted recessive. The variety in shape of the termination of the leaf is represented in these leaves.

## PLATE V.

- Fig. 5. Entire-leaved young plant of the  $F_2$  generation.
- Fig. 6. Incised-leaved young plant of the  $F_2$  generation.
- Fig. 7. Leaves of incised-leaved young plants of the  $F_3$  generation (from plant 29) showing the early definition of the character.
- Fig. 8. Leaves of incised-leaved young plants of the  $F_3$  generation (from plant 26) showing the longer retention of a partially entire margin in some of the leaves.
- Fig. 9. Entire leaves of young plant from isolated prothallus; presumably pure dominant.
- Fig. 10. Incised leaves of young plant from isolated prothallus; presumably pure recessive.



## GENETIC STUDIES IN POTATOES; THE INHERITANCE OF IMMUNITY TO WART DISEASE.

By R. N. SALAMAN, M.A., M.D., AND J. W. LESLEY, M.A.

A PRELIMINARY account of the work undertaken by the joint authors to elucidate the inheritance of immunity to wart disease by the potato was read before the International Potato Conference in November 1921. Since then another year's results are to hand, and the combined results are such as to support in general the views put forward on that occasion. Notwithstanding the fact that unresolved difficulties still present themselves, the importance of the problem under investigation is such that it seems desirable to put forward the facts and the conclusions to which they have led, without further delay. This course seems the more advisable when it is remembered that to carry out a test for wart on any particular desired mating requires at least three years to obtain a tentative result and a further year to confirm it.

It is generally recognised that once the immunity of a variety is established it has never been found to break down. The immunity is under the conditions of normal horticulture constant. Professor Schaffnit (1) has stated that degenerate plants of a hitherto immune variety will succumb to wart disease under normal conditions. We have found no evidence of such an occurrence during the ten years in which trials for wart immunity have been conducted at Ormskirk. Indeed, Sutton's Abundance has retained its immunity, under trial, for 14 consecutive years. We have, however, very frequently found rogues in immune stocks which have succumbed and which, had they not been identified by constant inspection during the growing period, would have been overlooked and given rise to just such a view as Professor Schaffnit advances. At Ormskirk it has been laid down that a trial in two consecutive years is necessary to determine the existence of immunity, and that the number of plants examined should not be less than forty. The experience we have gained undoubtedly supports this view, and although forty plants may be greater than is really essential, it is a wise measure of precaution. There are several conditions which affect the incidence of the disease in a susceptible variety. It is, above all, necessary that

the soil should be thoroughly infected with the spores of the pathogen, *Synchytrium endobioticum*, Schilb. Perc. This is assured at Ormskirk. The plants should be well grown and healthy. Infection with the virus diseases such as Leaf Roll and Mosaic, if severe, will produce such feeble plants with tubers so small and few that they may escape infection or at least the infection may escape detection. The effect of virus diseases is to hasten the maturity of the plant and this early ripening is to a considerable extent a protection against attack, especially in a cold and dry season. Not least important, as the season of 1921 taught, is that the rainfall should be normal, as moisture hastens and increases infection, whilst drought may so hinder the growth of the organism as to allow many susceptible plants to escape. This was exemplified in a very marked way in our cultures in 1921 and 1922. In the latter year all those which escaped infection in the abnormally warm dry season of 1921 were replanted and the result showed that many of those stocks grown in the open field which had so escaped fell readily to the disease in the cooler and moister season of 1922. On the other hand, certain extremely vigorous families were grown in 1921 in a small highly infected garden which, owing to trees and hedges and the very rich deep soil, had been protected from the intense heat and consequent evaporation which had taken place generally in the open in that year. In a family of 79 individuals, 76 were free from wart and not one of the 76 succumbed when grown in the trial grounds again in 1922. The families 318Bb/20 and 321Bb/20, both very vigorous, were also grown in this garden and although the non-warted seedlings of 1921 were not regrown in 1922, there is probably no reason to doubt the substantial accuracy of the figures then obtained. In the case of all the families which did not enjoy such exceptional advantages it was found that a considerable modification of the 1921 result was occasioned by the further test undertaken in 1922. The incidence of wart disease in 1922 was most severe and it is unlikely that the results obtained would be materially altered by a further year's test.

The series of families tested for their susceptibility or otherwise to wart disease arose from fertilizations made by hand under parchment paper bags and with all precautions. The families fall into six groups, seedlings of immune parents selfed, seedlings arising from the mating of two different immunes, seedlings of susceptible selfed parents, seedlings arising from the mating of two different susceptibles, seedlings of the mating immune by susceptible and, finally, seedlings of the mating susceptible by immune.



An examination of the tables reveals at once the fact that whereas an immune selfed or mated to another may produce, as found in one family, all immunes, it generally gives rise to a large majority of immunes and to a minority of susceptibles. On the other hand, susceptibles, with one notable exception, give rise to families composed entirely of susceptibles or at most contain a few per cent. of non-warted individuals. It can be accepted at once that immunity is dominant and susceptibility recessive. This view is directly opposed to that of Collins (2) who, however, at that time was not in possession of any consistent body of first hand facts, but it is in agreement with the indications obtained by Orton and Weiss (3).

Table II, giving the mating of the two immunes, Kerr's Pink and Champion the Second, deals with a family of 79 individuals who have been tested thoroughly in two consecutive years and the result may be taken as absolutely reliable. The ratio of 3 susceptible to 76 immune suggests at once a 1:15 ratio brought about by two factors independently capable of inducing immunity. We may call these factors *X* and *Y*. The existence of a 1:15 ratio is also supported by the evidence afforded in the mating of the Flourball  $F_2$  seedling (3 *Cb*23) Table I.

TABLE I.

*Immune Selfed.*

Name	Class Number	Number of Seedlings Tested	Non-warted	Susceptible	Dead
Flourball Seedling ...	3 <i>Cb</i> /19, 6 <i>Cb</i> /21	10	6	3	1
" " ...	3 <i>Cb</i> /19, 23 <i>Cb</i> /21	26	22	3	1
Leinster Wonder ...	307 <i>Bb</i> /20	10	5	5	0
" " ...	307 <i>Cb</i> /20	8	5	3	0
Leinster Wonder Seedling	307 <i>Cb</i> /20, 4 <i>Cb</i> /21	12	9	0	3
*Golden Wonder $\times$ Leinster Wonder $F^2$ Seedling }	305 <i>Cb</i> /20, 125/21	9	9	0	0
Majestic ...	309 <i>Bb</i> /21	25	10	9	6
Edzell Blue ...	302 <i>Bn</i> /20	13	12	1	0
" " ...	302 <i>Cn</i> /20	15	10	5	0
Edgecote Purple $\times$ Edzell Blue Seedling }	315 <i>Cb</i> /20, 31 <i>Cb</i> /21	13	10	3	0

\* Only this relatively small proportion of the family was tested.

TABLE II.

*Immune  $\times$  Immune.*

Name	Class Number	Number of Seedlings Tested	Non-warted	Susceptible	Dead
Kerr's Pink $\times$ Champion II ...	306 <i>Bb</i> /20	79	76	3	0
*Golden Wonder $\times$ Leinster Wonder	305 <i>Cb</i> /20	27	18	9	0

\* Only this relatively small proportion of the family was tested.

The late John Snell (4) undertook some experiments on the inheritance of wart in 1919. The seedlings were raised from natural berries. The notes containing Mr Snell's observations came into the hands of Mr W. Cuthbertson, from whose published letter the following facts are taken. The results from the following selfed plants, viz. Priory Queen, Favourite and Admiral, all synonyms of and doubtless identical with Abundance, showed 7 to be warted out of 80, figures again suggesting a 1:15 ratio. Possibly also Flourball is an  $XxYy$  plant because we have seedlings of it giving indications of both a 1:3 and a 1:15 family ratio derived from it. (See Table I.) In addition to an immune plant having a formula  $XxYy$ , the selfed immunes in Table I give at least one clear example of a 3:1 ratio—such immunes having the formula  $Xxyy$  or  $xxYy$ . Whilst the families of pure immunes derived from Leinster Wonder  $F_2$  and Golden Wonder  $\times$  Leinster Wonder  $F_2$  seedlings, though small, are evidence of some weight in favour of the existence of a homozygous immune plant, none of the six crosses of immune and susceptible produce a family of all immunes which points to the relative rarity of the homozygous immune parent.

If we regard the families 307 *Cb*, 4 *Cb* and 305 *Cb* 125 as homozygous types they would presumably have the formula  $XXYY$ . To these may be added the types  $Xxyy$  or  $xxYy$  which have already been identified.

There are, however, two immune parents which do not fit into this scheme, viz., Majestic and Leinster Wonder. The family of Majestic tested at Ormskirk in 1922 produced 10 immunes to 9 susceptibles. These numbers would not by themselves carry very much weight were it not that in the notes of the late J. Snell already alluded to, Majestic seedlings obtained from natural berries behaved in an almost identical manner, viz. 38 non-warted to 22 susceptible. The combined numbers would be 48:31, a close approximation to a 9:7 ratio which is not to be expected from the presence of two factors either of which, acting independently, can confer immunity. Similarly, Leinster Wonder selfed produced 10 non-warted to 8 susceptible individuals, a result which, in view of the Majestic findings, must be considered of some significance. The deviations from a 3:1 ratio are approximately three times the standard error which, together with the fact that both families exhibit ratios which approximate to 9:7, suggests the existence of two factors which produce immunity only when both are present. If, therefore, adhering to the two factors  $X$  and  $Y$  which independently were assumed capable of inducing immunity to wart, we make the further assumption that in the absence of a complementary factor, which we will call  $Z$ ,

neither  $X$  nor  $Y$  alone can induce immunity but that the combined action of either  $X$  or  $Y$  with  $Z$  will induce immunity equally as does the combination of  $X$  and  $Y$  together without  $Z$ , then a plausible explanation is found. Under such an hypothesis a plant heterozygous for  $X$  and  $Y$  and homozygous for the absence of  $Z$  would produce 9 immune to 7 susceptible plants.

Susceptible plants as judged from the selfed families, Table III, and the crosses of susceptible by susceptible, Table IV, would appear to be

TABLE III.

*Susceptible Selfed.*

Name	Class Number	Number of Seedlings Tested	Non-warted	Susceptible	Dead
Congo $\times$ Flourball $F_2$ Seedlings	K 23 Cb/19, 23 Cb/21	16	0	10	6
Edgecote Purple ... ..	147 Bb/20	31	2*	27	2
Myatt's Ashleaf ... ..	333 Bb/21	22	11	9	2
„ „ ... ..	333 Cb/21	28	11	17	0

\* Very poor small plants : probably too feeble to have contracted the disease.

TABLE IV.

*Susceptible  $\times$  Susceptible.*

Name	Class Number	Number of Seedlings Tested	Non-warted	Susceptible	Dead
Congo-Flourball Seedling $\times$ Edgecote Purple	330 Bb/21	14	0	14	6
May Queen $\times$ Edgecote Purple	332 Bb/21	33	1	31	1
Eclipse $\times$ Sharpe's Victor	326 Bb/21	6	0	6	0
Myatt's Ashleaf $\times$ Edgecote Purple	334 Bb/21	22	2	20	0
Edgecote Purple $\times$ Edgecote Purple	334 Cb/21	37	9	28	0
Edgecote Purple $\times$ Myatt's Ashleaf	338 Bb/21	39	5	31	3
	338 Cb/21	53	8	43	2

divided into those which produce only susceptibles and those which produce a considerable number of immunes also. This latter group is seen to include the selfed and crossed families in which Myatt's Ashleaf is a parent. The former group includes families in which an occasional immune is noted, viz., in Edgecote Purple 147 Bb/20 where there are 27 susceptibles and 2 immunes, and in May Queen by Edgecote Purple where there are 37 susceptibles to 1 immune. In the former family the immune plants are very poor ones and may be left out of consideration,

and in the latter, although the immune plant was not abnormal in growth it is not improbable that its immunity in 1922 is due to some fortuitous circumstance, and that it will not be maintained.

When susceptibles are crossed to immunes or vice versa the results are again of two kinds, a larger class where the resultant family is made up of equal numbers of immune and susceptible individuals, and another of which we have but one example, viz. Arran Rose  $\times$  Sharpe's Victor (324 Bb/21, Table V), where there is a large majority of immunes.

TABLE V.

*Immune  $\times$  Susceptible.*

Name	Class Number	Number of Seedlings Tested	Non-warted	Susceptible	Dead
Arran Rose $\times$ Sharpe's Victor	324 Bb/21	16	11	4	1
Witchhill* $\times$ Myatt's Ashleaf	316 Bb/20	25	9	16	0
Snowdrop* $\times$ Myatt's Ashleaf	316 Cb/20	32	16	16	0
Edzell Blue $\times$ Edzell Blue	313 Cb/20	25 25	14 14	9 9	2
Edgescote Blue $\times$ Edgescote Purple	304 Bb/20	27	11	16	0
Edzell Blue $\times$ Edzell Blue	304 Cb/20	38	21	17	0
Myatt's Ashleaf $\times$ Myatt's Ashleaf	315 Cb/20	28 28	16 16	12 12	0
Myatt's Ashleaf $\times$ Myatt's Ashleaf	303 Cb/21	44	23	21	0

\* Synonyms for one and the same plant.

The susceptible individuals who on selfing produce no immunes and behave in crossing as pure recessives must, on the  $X$  and  $Y$  hypothesis be represented as  $xx yy$ , whilst the immune parents with which they are mated will be examples of the  $Xx yy$  or  $xx Yy$  types of immune plants when the resultant family consists of half immune and half susceptible plants.

In Tables V and VI there are several examples which illustrate this mating, but attention is in particular drawn to the mating 316/20 and

TABLE VI.

*Susceptible  $\times$  Immune.*

Name	Class Number	Number of Seedlings Tested	Non-warted	Susceptible	Dead
Kew 2 $\times$ E. Regent 63, 78 $\times$ Shamrock	318 Bb/20	38	17	21	0
Kew 3 $\times$ K 23, 17 $\times$ Leinster Wonder	321 Bb/20	37	15	22	0

313/20. In these examples Myatt's Ashleaf is used to cross Witchhill and Snowdrop. The resulting families were identical in appearance and are seen to be similar also in their reaction to wart disease. It was further found also that their heritable cropping capacity as exhibited by the graphs of the curves of their offsprings' crops was also identical<sup>1</sup> (5). It is well known that Witchhill and Snowdrop, though considered by most experts to be but one and the same variety—an example of synonymity amongst potatoes—are by others still regarded as distinct. We are now in a position to say that not only are these two varieties identical morphologically, but that in respect to two most important physiological reactions, viz. that of immunity to wart disease and heritable cropping capacity, they are also identical.

It may here be pointed out that the reciprocal crosses Myatt's Ashleaf and Edgecote Purple (Table IV) are also identical in respect to their susceptibility to wart disease. Indeed, identical results from the reciprocal crosses 304 *Cb*/20, 304 *Bb*/20 and 315 *Cb*/20 were obtained, which would tend to confirm the view that there is no linkage or other effect induced by sex on the inheritance of immunity in the potato.

The families in which Myatt's Ashleaf enter differ in their reaction to wart disease.

Whilst other susceptible plants which have been tested appear to give all warted offspring on selfing, Myatt's Ashleaf produces a family in which the ratio of susceptible to non-warted is 26 : 22. Further, in its matings with the susceptible Edgecote Purple, the resultant family consists of 122 susceptible individuals and 24 immunes, a result deviating from a 3 : 1 ratio by twice the standard deviation.

Both results suggest that Myatt's Ashleaf is really an immune variety whose immunity is being held up by one or more inhibiting factors. Indeed, in a previous publication (5) evidence was adduced that Myatt's Ashleaf contained a factor "*B*" which inhibits its own immunity factor *Y*. However, the ratio 26 : 22 in the selfed family now available indicates a 9 : 7 ratio, and seems to suggest that there may be not one, but two inhibitors, *A* and *B*, existent in an heterozygous state. With the existing data, the relation of these inhibitors with the factors *X*, *Y*, and *Z* cannot be further elucidated but it may well be that it is necessary for both of them to be present to inhibit either of the immune factors, though their combined presence is incapable of inhibiting the immunity conferred by the combined presence of both *X* and *Y*. On such an hypothesis Myatt's Ashleaf would have the formula *xx YY Aa Bb*.

<sup>1</sup> This refers to an unpublished work on the *Inheritance of Cropping*.

In Snell's notes are given the results of a test on 29 seedlings of a natural ball of President, with a result that 15 are susceptible and 14 not, which suggests that President is a susceptible variety similar in kind to Myatt's Ashleaf.

It has been seen that the mating of Myatt's Ashleaf  $\times$  Edgescote Purple results in the production of 122 susceptible to 24 immune plants. This ratio is suggestive of 3:1, although its deviation is just over twice the standard error. If the formula  $xxYYAaBbZZ$  be adopted for Myatt's Ashleaf, the corresponding formula for Edgescote Purple would be  $xyyyAaBBZZ$ , which would allow of 3 susceptible to 1 immune in crossing. The formulae both for Edzell Blue and Witchhill might be  $xxYyaaBBZz$ , which would allow for the production on crossing with Myatt's Ashleaf of a family composed of one half immune and one half susceptible seedlings.

The crosses in Table VI representing the matings of susceptibles by immunes present two results which may be interpreted as follows:

The first family 318  $Bb/20$  where the immune parent is Silver Shamrock produced 17 non-warted to 21 susceptible which is near enough to 19:19 or equality. This would be a probable result of the mating of a susceptible variety not containing  $X$  or  $Y$  or the inhibitors to an immune which is of the 3:1 type. Silver Shamrock, which is a Flourball seedling, is thus not unlikely to be just such a type of immune.

The second family 321  $Bb/20$  is crossed by Leinster Wonder which, it has been seen, is probably to be regarded as devoid of the complementary factor  $Z$  and as bearing the formula  $XxYyzz$ . If the susceptible is  $xxYyzz$  and Leinster Wonder be as suggested then a ratio of 3 non-warted to 5 susceptible would result; actually in the family 321  $Bb$  the ratio is 15 immunes to 22 susceptibles; on a 3:5 basis the number would be 14:23.

Sharpe's Victor in its reaction presents a further problem; itself it is susceptible but nevertheless offers sufficient resistance to wart disease as for some time to have been considered as an immune. The small family Eclipse  $\times$  Sharpe's Victor is all warted, but the mating Arran Rose (immune)  $\times$  Sharpe's Victor (Table V) gives rise to a family of 11 immunes to 4 susceptibles, an excess of immunes which suggests a 3:1 ratio. A result which might arise where Arran Rose owes its immunity to one of the  $X$  or  $Y$  factors, and Sharpe's Victor to contain the other but in an inhibited combination due to the combined presence of  $A$  and  $B$ .

It would appear that certain plants may offer a varying degree of resistance to the attacks of the pathogen, which is very possibly correlated with their genotypic composition. This resistance is, however, insufficient to protect the plant against the full force of a heavy attack, such as befell the plants at Ormskirk in 1922. On the other hand a plant which owing to its genotypic character is immune, remains constantly so under all the conditions of experimentation as yet employed. Indeed, a suggestion of the kind has been made by Orton and Weiss who state that "such differences in the degree of resistance or susceptibility shown are believed to be inherent in the variety itself and this would indicate that the extremes of resistance and susceptibility are dependent for their expression upon more than a single factor difference." The evidence we have brought forward as a result of our researches at Ormskirk would seem to afford the necessary proof of this contention, for whether we explain our facts by supposing that some plants might be susceptible because they have no immunity factors, or susceptible because the immunity factors are held in suspense by inhibitors, or susceptible because the immunity factor, though present, lacks its complementary factor, the essential fact remains that there is unmistakable evidence, that certain susceptible plants, such as Myatt's Ashleaf and Sharpe's Victor, behave quite differently both when selfed and when crossed as compared to other immunes.

We may summarise our results as follows:

(1) That immunity to wart disease in the potato is dependent upon segregating factors.

(2) That immunity is dominant to susceptibility: though this dominance may be inhibited by other factors.

(3) That there are at least four types of immunes, which may be described as:

(a) pure immunes,

(b) immunes which, on selfing, give 15 immunes to 1 susceptible,

(c) " " " " " 3 " " 1 "

(d) " " " " " 9 " " 7 "

and that the immunity they respectively possess may be due to the presence of one or more immune factors, the evidence for the co-presence of at least two immune factors in some varieties being particularly strong.

(4) That susceptibles may be of various sorts:

- (a) due to the absence of either of the immune factors  $X$  or  $Y$ ,
- (b) due to the absence of the complementary factor  $Z$ , though either  $X$  or  $Y$  may be present,
- (c) due to the presence of an inhibitor of the immunity factor.

(5) Difference of genotype amongst immune plants is not reflected by any difference of degree in the immunity conferred. Difference of genotype, however, amongst susceptibles does appear to confer considerable differences in degree of susceptibility. However, under the conditions of field experimentation the line between immune and susceptible once reached is absolute.

(6) No differences were discovered as regards the immunity in respect to reciprocal crosses.

(7) There is no evidence of any relation or linkage between wart disease and any other character of the plant.

We take this opportunity of thanking Mr Heber Smith for having carried through the 1922 field work at Ormskirk, and Mr H. Bryan, Superintendent of the National Institute of Agricultural Botany Potato Testing Station, for his assistance, and to the Ministry of Agriculture which has kindly allowed all the material to be tested free of charge.

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# SPECIES-CROSSES IN *COCHLEARIA*, WITH A PRELIMINARY ACCOUNT OF THEIR CYTOLOGY

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(With Plates VI—IX and One Text-figure.)

## I. SPECIES-CROSSES IN *COCHLEARIA*.

BY M. B. CRANE.

THE original plants of *Cochlearia officinalis* and *Cochlearia danica* used in the following experiments were collected by Mrs Bateson at St David's off the S.W. coast of Wales, and those of *Cochlearia anglica* by Miss D. M. Cayley at Hayling Island, Hants.

The plants of *danica* were very uniform, and natural seeds gathered from them gave plants that were uniform in all characters. In our experience *danica* always set seed with perfect freedom when selfed, and subsequently several hundred selfed seedlings were raised. With one exception they were remarkably uniform. The exceptional plant was a dwarf; it had very dark green fleshy leaves, and in all parts was very small: see Plate VI, fig. 2.

The leaves of some of the *anglica* plants were slightly longer and more attenuated at the base than those of others; they also varied in anthocyanin colour. Over 100 selfed seedlings were raised from two plants, and among them the above differences again occurred.

The collected plants of *officinalis* varied in size and in anthocyanin colour. The same variations occurred in natural seedlings raised from them. A few of these natural seedlings were protected and intercrossed, and plants were raised from the seed thereby obtained. These plants although variable did not widely differ. Two of them, one designated "A" and the other "B," were selfed. They proved to be only partially self-fertile, but over 200 seeds were obtained. These plants appear as "A" and "B" respectively throughout this paper, and are later referred to

in greater detail. To all outward appearance they were identical, and in all characters they appeared to be typical *officinalis*. In a family raised from plant "A" selfed, the majority of the plants are similar to "A" in size, but a few are smaller. No other difference is noticeable in this family at the present time. Selfed plants raised from "B," however, vary somewhat widely in several characters, and one has curiously folded leaves like those in Fig. 3.

*Description of Species.*

In the following descriptions only the most salient characters, and those relating to the present preliminary account of the seedlings raised from them, are described.

*C. officinalis* Linn.

*Radical leaves*: roundish or reniform, deeply cordate at the base and generally entire: petioles longer than *danica*.

*Stem leaves*: sessile, amplexicaul (except the lower ones which are stalked), angulated, with a few large teeth or lobes, occasionally entire.

*Branches*: ascending, up to 2' long: the primary habit is erect, but as the flowers, leaves and lateral growth develops, they recline and become spreading.

*Capsules*: sub-globular.

*Size*: in all parts large: much larger than *danica*. Cf. Figs. 1 and 4.

The whole plant is deep green: paler than *danica*, but much deeper than *anglica*.

*C. danica* Linn.

*Radical leaves*: roundish or angular (the primary leaves are roundish, but the later ones are angular), deeply cordate at the base: petioles shorter than *officinalis*.

*Stem leaves*: stalked (occasionally the uppermost ones are sessile), 3 to 5-lobed, somewhat resembling those of the Ivy.

*Branches*: central stem erect and very short, lateral branches decumbent, 3" to 9" long: in general the habit is very spreading and prostrate.

*Capsules*: ovoid.

*Size*: in all parts small.

The whole plant is dark green.

*C. anglica* Linn.

*Radical leaves*: oblanceolate or oval, attenuated or rarely rounded (but never cordate) at the base, usually entire, but occasionally toothed.

*Stem leaves*: generally sessile, semi-amplexicaul, rhomboidal-oblong or strap-shaped, usually toothed.

*Branches*: ascending, primarily erect, 3" to 1' long: central stem shorter and more erect than the lateral branches.

*Capsules*: oval, much compressed, the replum being 4 to 6 times as long as broad.

*Size*: style longer and flowers much larger than those of *officinalis*.

The whole plant is pale green.

In 1921 crosses were made by Mr H. W. Jack between *officinalis* and *danica* and the following families raised:

Fam. 1/21 *officinalis* × *danica*, 5 seeds set, 5 germinated

„ 2/21 *danica* × *officinalis*, 13 „ „ 10 „

Differences in size and in anthocyanin colour occurred in both families. The variation in size however was not wide. In habit of growth and all other characters the plants were uniform, and the following description applies to all.

*Description of  $F_1$ , *officinalis* × *danica* and *danica* × *officinalis*.*

*Radical leaves*: roundish or angular (the primary leaves roundish and the later ones angular), deeply cordate at the base: petioles intermediate between parents in length.

*Stem leaves*: stalked (occasionally the uppermost are sessile), 3 to 5 lobed, resembling those of the Ivy.

*Branches*: central stem erect, short but much longer than that of *danica*, lateral branches decumbent, 6" to 18" long: in general the habit is spreading and prostrate.

*Capsules*: sub-globular.

*Size*: in all parts intermediate between parents.

*Colour of leaves*: intermediate, lighter than *danica*, darker than *officinalis*.

In general habit and leaf shape they resemble *danica*. The capsules are similar to those of *officinalis* in shape. In size and foliage colour they were intermediate: see Figs. 1 and 4.

Three plants from family 1/21 and five from 2/21 were used in further breeding experiments. The pollen of the  $F_1$  plants varied, but in all a considerable proportion was aborted and obviously useless. Varying degrees of self-fertility also occurred among the plants, but this was not associated with the variation in the pollen. Among the plants of typical *danica* and *officinalis* used in the experiments an obviously aborted

pollen grain has been but rarely observed. The approximate proportion of good pollen and the percentage of seed germination of the  $F_1$  plants that have been used in further breeding, and of the parents, are given below.

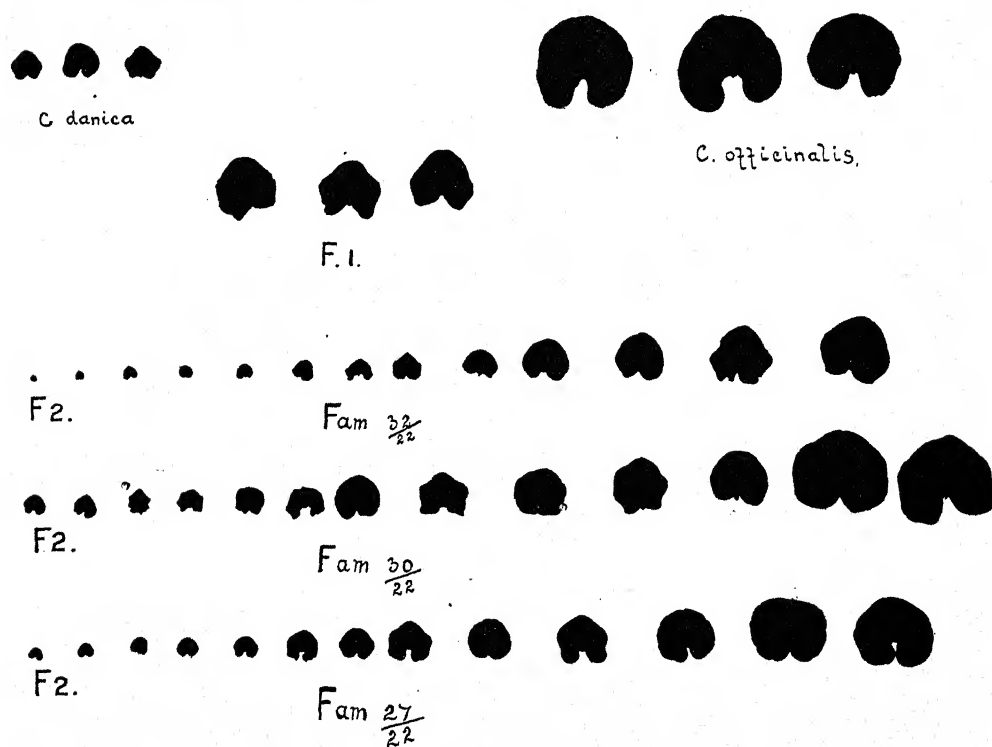
Family Number	Parentage	Pollen percentage apparently good	Number of seeds sown	Number of seeds germinated	Percentage germinated
1/22	<i>C. danica</i>	100	120	120	100
2/22	<i>C. officinalis</i>	100	125	106	84.8
25/22	"B" × 1 <sup>1</sup> /21	—	16	13	81.2
26/22	1 <sup>1</sup> /21 (selfed)	76.0	305	189	61.9
10/22	" × <i>danica</i>	—	30	21	70.0
18/22	" × "B"	—	16	11	68.7
27/22	1 <sup>2</sup> /21 (selfed)	34.0	245	144	58.7
11/22	" × <i>danica</i>	—	44	23	56.8
19/22	" × "B"	—	55	26	47.2
28/22	1 <sup>2</sup> /21 (selfed)	72.0	31	22	70.9
12/22	" × <i>danica</i>	—	12	10	83.3
20/22	" × "B"	—	8	5	62.5
29/22	2 <sup>1</sup> /21 (selfed)	83.0	207	137	66.1
13/22	" × <i>danica</i>	—	84	67	79.7
21/22	" × "B"	—	41	28	68.2
30/22	2 <sup>2</sup> /21 (selfed)	31.0	240	134	55.8
14/22	" × <i>danica</i>	—	22	16	72.7
22/22	" × "B"	—	2	1	50.0
31/22	2 <sup>3</sup> /21 (selfed)	65.0	160	75	46.8
15/22	" × <i>danica</i>	—	6	5	83.3
23/22	" × "B"	—	3	1	33.3
32/22	2 <sup>5</sup> /21 (selfed)	59.0	300	218	72.6
16/22	" × <i>danica</i>	—	13	11	84.6
24/22	" × "B"	—	14	9	64.2
—	2 <sup>6</sup> /21	51.0	—	—	—
17/22	" × <i>danica</i>	—	34	27	79.4

## $F_2$ .

919  $F_2$  plants were raised of which 850 survive. Among them remarkably wide variations occur. Plants which appear to be identical with *danica* but rarely occur, and plants which appear typically *officinalis* although more frequent are not common. Many individuals are in all parts very much smaller than *danica*, but none are larger than the *officinalis* originally used in the experiments. In certain families the size of the plants swings more towards *danica* than *officinalis*; others swing more towards *officinalis*. The actual range of the variation is, however, almost equally wide in all families, but in some it begins much below *danica* and ends considerably below *officinalis*, whereas in others it begins approximately at *danica* and goes up to *officinalis*: see text-figure 1.

That the original plants of *officinalis* used in the experiments were not homozygous for size was subsequently evident in their selfed offspring, and to this the above differences in separate  $F_2$  families are probably attributable.

Various recombinations of characters, and plants which are very distinct from either parent occur. That the variation in size goes below *danica* but not above *officinalis* has previously been mentioned. In most characters, however, the range of the variation extends considerably beyond both parents, but in all numerous quantitative differences occur, and it is not possible at the present stage of the experiments to classify the plants with any degree of certainty. A fuller account of the varia-



Text-figure 1. Typical radical leaves of *danica*, *officinalis*,  $F_1$  and  $F_2$  plants. In all cases they were the largest leaves on the plants. In each of the  $F_2$  families the largest leaf from 13 individuals is shown; on the left is the largest from the smallest plant, and on the right the largest from the largest plant in the family. The others are various sizes which occur in between the extremes.

Note. In Fam. 32 the size of the leaves begins far below *danica* and ends below *officinalis*. In Fam. 30 they begin approximately at *danica* and end at *officinalis*.

tions which occur in these families will be prepared when the work has been carried further.

In addition to the plants which from cytological and genetic evidence we may regard as true *officinalis* and *danica* respectively, e.g., the actual

parents of families 1/22 and 2/22, the previously mentioned plant "B" was introduced into the crossing experiments as a supposed *officinalis*. It has been shown that this plant came from a wild *officinalis*, and to the eye it was *officinalis*. However, as is later shown by Miss Gairdner, it proved to have 30 chromosomes instead of 28, and among its selfed offspring and also in a family raised from crossing it with true *danica*, there appeared plants with folded leaves like those in Fig. 3. Other wide variations also occurred in these families.  $F_1$  plants from true *officinalis*  $\times$  true *danica* are comparatively uniform, and as would be expected such wide variations do not appear until  $F_2$ . There is therefore a good deal of evidence from which it is tempting to assume that plant "B" was not a true *officinalis*, but a cross-bred plant, presumably a derivative of *officinalis*  $\times$  *danica*. It was used in various matings under the impression that it was an *officinalis* like others, but the crosses in which it occurs are not the back crosses which in the design of the experiments they were intended to be. The results, however, show many points of interest, and from what is now evident such plants as "B" would almost be expected in our original material, collected as it was from a locality where *officinalis* and *danica* grow together.

#### Back crosses.

From  $F_1 \times$  plant "B," 94 plants were raised and 81 survived. In size they vary from plants as large as *officinalis*, to plants smaller than the  $F_1$ , but none are as small as *danica*. The whole of the plants, however, have many characteristics of *officinalis*, and a much greater resemblance to it than to either *danica* or the  $F_1$ . Even the smallest would be described as small plants of *officinalis*, and would not be confused with *danica*.

From  $F_1 \times$  *danica* 180 plants were raised of which 170 survived. None were smaller than *danica* and only one was larger than the  $F_1$ . Many plants appear to be identical with *danica*, and a few with the  $F_1$  plants, but the majority are intermediates, which in all characters except size are very much like *danica*.

In 1922 crosses were made between *C. anglica* and *C. officinalis* and the following families raised:

Fam. 7/22,	<i>anglica</i> $\times$ <i>officinalis</i> ,	13 seeds set,	10 germinated
" 8/22,	<i>officinalis</i> $\times$ <i>anglica</i> ,	6 " "	3 " "

Preliminary attempts to cross *anglica* with *danica* failed.

In all characters *anglica* and *officinalis* widely differ. During the early stages of growth the  $F_1$  plants are very distinct from either parent,

see Fig. 4. Later, they resemble *anglica* much more than *officinalis*, but in all their characters intermediate properties are evident. The radical leaves vary, but they are all abrupt at the base. Occasional leaves, however, are toothed and abruptly attenuated, others are slightly cordate at the base, but none are attenuated like *anglica* or deeply cordate like *officinalis*. The leaves of some appear darker green than those of others, but this variation is associated with an anthocyanin pigment.

Further breeding with these plants is being attempted.

Since these experiments were begun several species and forms of *Cochlearia* have been obtained from various sources. Among them was a form which had leaves similar in size and shape to *officinalis*, but they were much paler green. The capsules were flattened and compressed along the replum, being somewhat intermediate between *anglica* and *officinalis* in shape. From one of these plants (No. 5/21) 45 selfed seedlings were raised; they slightly differed in size, but in leaf-colour and all other characters they were uniform.

Using 5/21 as the ♀ parent the following crossed families were raised:

Fam. 35/22. 5/21 × *danica*, 67 plants. These plants were very uniform and in all characters were intermediate between the parents.

Fam. 36/22. 5/21 × *officinalis*, 34 plants. In size these plants slightly differed, but in leaf-colour and all other characters they were uniform and intermediate between the parents.

Fam. 37/22. 5/21 × *anglica*, 28 plants. In all their characters intermediate properties are evident, but they resemble *anglica* more than *officinalis*.

Regarding the origin of 5/21 we have no knowledge, it was obtained with many similar plants from three Botanic Gardens as *anglica*. In general it is much nearer *officinalis* than *anglica*, but certain of its characters suggest that both species may be involved in its origin. At present, however, this cannot be seriously considered.

Among the plants that came with 5/21 was one (No. 6/21) that was in all characters intermediate between 5/21 and *danica*. Selfed seedlings raised from this plant varied in size from *danica* to 5/21, and in leaf-colour from pale to dark green. This is obviously an example of natural crossing.

It has previously been shown that the plants of *anglica*, collected at Hayling Island, varied but little, and that among their selfed offspring only minor differences occurred. Among a number of *anglica* plants

collected by Miss Cayley at Blakeney Point in Norfolk, however, considerable variation occurred. Some of these plants appear to be identical with the  $F_1$  plants we obtained by crossing *anglica* with *officinalis*; others are distinct from our  $F_1$ 's and also from *anglica*. At Blakeney Point *anglica* and *officinalis* both occur; at Hayling Island, however, Miss Cayley could not find *officinalis*, but only *anglica* and *danica*. Our own attempts to raise  $F_1$  plants by crossing *anglica* with *danica* have so far wholly failed, but  $F_1$  plants from *anglica*  $\times$  *officinalis* and *officinalis*  $\times$  *danica* have been freely obtained. It therefore seems clear that the variability of the *anglica* plants from Blakeney Point is due to the presence of *officinalis* and to natural crossing occurring between them. Those from Hayling Island varied but little, and as there were no *officinalis* in the locality, wide variation would not be expected.

In Sowerby's *English Botany*, 3rd Ed. Vol. i. the radical leaves of *anglica* are described as "attenuated or rarely rounded (but never cordate) at the base." Later, however, he states, "It is said sometimes to have the radical leaves cordate at the base, and if so, it may possibly be only another sub-species of *C. polymorpha*." It is of interest to notice that this latter description agrees more with the radical leaves of *anglica*  $\times$  *officinalis* derivatives, and it is tempting to assume that it is to such plants, and not to true *anglica*, that this early record relates.

From the great variety of forms of *Cochlearia* resembling *officinalis* and *danica* which are found in nature it is evident that between these species a certain amount of natural crossing occurs. Between them and their derivatives, however, and among the derivatives themselves, often but small quantitative differences occur. Forms which are closely allied therefore appear, and become confused.

The plants of *danica* collected from Hayling Island and from St David's were identical, and with the one exception previously mentioned, no variation occurred in selfed families raised from them. Prof. Udny Yule kindly sent us several plants of *danica* which he collected in Cornwall. These plants proved to be of three kinds: (1) identical with those from St David's, and Hayling Island; (2) a form in which the margin of the leaves folds upwards, the upper surface of the leaves being concave; (3) a form with slightly smaller leaves than the above forms, and with an even more prostrate habit of growth. Natural seeds taken from these three forms gave very uniform offspring.

For other species of *Cochlearia* recently brought into the experiments we are indebted to Mr G. C. Druce for seed of *C. micacea*, Marshall, and to Mr J. R. Matthews for plants of *C. alpina*, Watson.



## II. CYTOLOGY.

By A. E. GAIRDNER.

Study of the cytology of these very distinct forms of *Cochlearia* has elicited several points of interest.

So far only the chromosomes in the somatic tissue have been examined, and nothing is yet known about their behaviour in meiosis. The flowers are exceedingly small, as also are the germ cells, and consequently they are not very suitable material for a critical study of these phases.

The chromosome counts have been made exclusively from root tips fixed in strong Flemming with an equal quantity of distilled water, and stained by the iron-alum-haematoxylin method.

Seedlings got by self-fertilizing the original wild plants were examined first, and it was found that the somatic number of chromosomes in a plant of *C. officinalis* was 28, while the much smaller species *C. danica* had 42.

The chromosomes in *C. officinalis*, when well spread out in an equatorial plate, are approximately equal in shape and size, being two to three times as long as they are broad, and pointed at the ends (Plate IX, fig. 1). In *C. danica* on the contrary, though a proportion of the chromosomes are as large as those in *officinalis*, others are much smaller (Plate IX, fig. 2). It is generally possible to pick out 14 large and 14 small chromosomes in the *danica* plates, while the remaining 14 are intermediate in size.

Crosses were made between various plants of these two species and the somatic number of chromosomes in seven of the resulting  $F_1$  plants was found to be 35—36. So far as one can make out only 14 to 16 of these are the large type, the reciprocal crosses being identical (Plate IX, figs. 7, 9).

Many  $F_2$  plants have now been examined and they range from the 28 chromosomes of *C. officinalis* up to 40, and it is probably only accidental that no plant with the whole 42 of *C. danica* has yet been found. Fourteen is the most usual number of large chromosomes, but in the smaller plants there may be only twelve large chromosomes and in the larger plants 16 of these. In some cases pairing between homologous chromosomes is plainly seen. On comparing Table III with Plate VIII, though at a first glance the number of chromosomes seems to increase as the size of the plant decreases, it will be found on further examination that no real correlation between the two exists. Any effect the

chromosomes may have upon the size of the plant cannot be due to their number alone.

As stated by Mr Crane the plant "B" which was used in many of the crosses in 1922 proved to be peculiar. No satisfactory cytological material was got from it, but two of its progeny had 28 and 34 chromosomes respectively, and from other evidence "B" is thought to have had 30. Back crosses with the two parent types gave a wide range of chromosome numbers, as will be seen by the Tables IV and V.

The *C. anglica* specimens from Hayling Island had 49—50 chromosomes, all being of the large *officinalis* type (Plate IX, fig. 3). Some of these plants were selfed and two of the resulting seedlings examined. One had 48 chromosomes while the other showed certainly 50, which suggests that these plants also were not pure. *anglica*  $\times$  *officinalis* gave the expected 39—40 (Tables I—VI).

The plant numbered 5/21 which was received from Mr Hales at the Chelsea Physic Garden, which is described above, had only 24—26 chromosomes, all of the large type (Plate IX, fig. 5). Nothing is known of the antecedents of this plant but it appears to have been of hybrid origin, as two plants got from it by self-fertilization had 28 and 30 chromosomes respectively (Table VIII). A second plant 6/21 received from the same source had 33—35 chromosomes, at least 12 of these being of the small *danica* type.

Table VII gives the chromosome numbers of some of the wild *anglica* plants collected at Blakeney Point.

The best known examples of dicotyledons in which 7 is the basal number of chromosomes are the species of *Oenothera* and *Rosa*, the *Leguminosae* also supplying several examples. According to the lists given by Tischler (1922) and Marchal (1920) this number has not so far been found among the *Cruciferae*. Marchal gives 9 species of this genus as having 8 chromosomes as their basal number, to which must be added *Stenophragma Thalianum* and *Brassica campestris* with 5—10 and *Lunaria biennis* with 12, showing that there is no fixity in the number throughout the Order.

In the present state of our knowledge little can be said as to how these different forms have arisen. Marchal (1920) suggests that an increase in chromosome numbers is probably produced by fractioning, and the small size of some of those in *danica* is consistent with this view. Only a few measurements have been taken, but no obvious increase in the size of the nucleus has been observed where the number of chromosomes is highest. *C. alpina*, which has only 28 chromosomes,

has been compared with one of our hybrid plants of about the same size, having 37, and the measurements of cell and nucleus were the same. *C. officinalis* and *C. anglica* have also been compared with the same result.

It will be noticed that the  $F_1$  plants having 35 chromosomes have given rise to plants with 40.

Blackburn and Harrison (1921) and Täckholm (1922) found that in hybrids between roses with differing numbers of chromosomes there was only a partial reduction in the heterotype division, a number of chromosomes remaining as univalents and either going entire to the poles or dividing, sometimes at the heterotype sometimes at the homotype division. In this way germ cells are formed having considerably more chromosomes than the haploid number of the parent plant. Presumably the same thing is happening in *Cochlearia* and it is hoped in time to get cytological evidence on the subject. It has become plain that crossing takes place to a considerable extent in nature between the various species of *Cochlearia*, and more work must be done to disentangle pure lines from the material we have at present.

In their remarkable studies of *Datura* Blakeslee (1922) and Belling identify particular plant forms with special chromosome configurations. This in our work has not up to the present been found possible.

TABLE I.

Cochlearia species	Number of Chromosomes
<i>C. officinalis</i> , plant "A" ...	28
<i>C. danica</i> , 4 plants ...	42
<i>C. anglica</i> , several plants ...	49—50
<i>C. alpina</i> ... ..	28
<i>C. micacea</i> , 2 plants ...	34—36

TABLE I A.

Cochlearia	Number of Chromosomes
Plant "B" ...	? 30
" selfed, 1922 plants	
2 <sup>1</sup> ...	34
2 <sup>2</sup> ...	28
<i>C. anglica</i> , selfed 5 <sup>4</sup> ...	50
" " 5 <sup>3</sup> ...	48

TABLE II.

<i>C. officinalis</i> × <i>danica</i> and recip.	Number of Chromosomes
<i>C. officinalis</i> × <i>danica</i> , 1921 plants 1 <sup>2</sup>	35
" " " 1 <sup>5</sup>	35
<i>C. danica</i> × <i>officinalis</i> " 2 <sup>5</sup>	35
" " " 2 <sup>6</sup>	35
" " " 2 <sup>9</sup>	35
" " " 2 <sup>10</sup>	35—36

TABLE III.

<i>F<sub>2</sub></i> from <i>C. officinalis</i> × <i>danica</i>			Number of Chromosomes
2 <sup>2</sup> /21 selfed, 1922 plants	30 <sup>1</sup>		28
" "	30 <sup>2</sup>		36
" "	30 <sup>3</sup>		33—34
" "	30 <sup>4</sup>		39—40
" "	30 <sup>5</sup>		35—39
" "	30 <sup>6</sup>		39—40
" "	30 <sup>7</sup>		35
" "	30 <sup>8</sup>		39—40
" "	30 <sup>9</sup>		40+
" "	30 <sup>10</sup>		36—37
" "	30 <sup>11</sup>		38
" "	30 <sup>17</sup>		32
2 <sup>5</sup> /21 selfed	32 <sup>1</sup>		32—34
" "	32 <sup>2</sup>		31—32
" "	32 <sup>3</sup>		31—33
" "	32 <sup>5</sup>		37
" "	32 <sup>6</sup>		31—33
" "	32 <sup>8</sup>		38
" "	32 <sup>9</sup>		37
1 <sup>2</sup> /21 selfed	27 <sup>1</sup>		30

TABLE IV.

<i>F<sub>1</sub></i> ( <i>officinalis</i> × <i>danica</i> ) × plant "B" and recip.			Number of Chromosomes
1 <sup>2</sup> /21 × plant "B", 1922 plants	19 <sup>1</sup>		33—35
" "	19 <sup>3</sup>		33—35
" "	19 <sup>4</sup>		31—33
" "	19 <sup>5</sup>		28
Plant "B" × 1 <sup>1</sup> /21	25 <sup>1</sup>		39—40
" "	25 <sup>4</sup>		35
" "	25 <sup>5</sup>		33—35
" "	25 <sup>8</sup>		33—35
" "	25 <sup>9</sup>		38—40
" "	25 <sup>7</sup>		40

TABLE V.

<i>F<sub>1</sub></i> × <i>danica</i>			Number of Chromosomes
2 <sup>1</sup> /21 × <i>danica</i> , 1922 plants	13 <sup>1</sup>		40—41
" "	13 <sup>4</sup>		37—39
1 <sup>2</sup> /21 × <i>danica</i>	11 <sup>1</sup>		38
" "	11 <sup>3</sup>		36—38

TABLE VI.

<i>C. officinalis</i> × <i>C. anglica</i> and recip.			Number of Chromosomes
<i>C. officinalis</i> × <i>anglica</i> , 1922 plants	7 <sup>1</sup>		39—40
" "	7 <sup>3</sup>		40
<i>C. anglica</i> × <i>officinalis</i>	8 <sup>1</sup>		39
" "	8 <sup>2</sup>		40

TABLE VII.

<i>C. anglica</i> , wild plants from Norfolk			Number of Chromosomes
Plant "A"	...		37
" "B"	...		42—44
" "C"	...		36—37
" "D"	...		40—42

TABLE VIII.

Large plant from Chelsea Physic Garden		Number of Chromosomes
5/21...	...	24—26
5/21 selfed, 1922 plants	34 <sup>1</sup>	30
" " "	34 <sup>2</sup>	28
5/21 $\times$ <i>danica</i> "	35 <sup>1</sup>	34—35
5/21 $\times$ plant "B", 1922 plants	36 <sup>1</sup>	28
" " "	36 <sup>2</sup>	28
5/21 $\times$ <i>anglica</i> "	37 <sup>1</sup>	35—36
6/21 smaller plant from Chelsea		33—35

## DESCRIPTION OF PLATES.

## PLATE VI.

- Fig. 1. *C. officinalis*, *C. danica*, *officinalis*  $\times$  *danica*, and *danica*  $\times$  *officinalis*  $F_1$  plants.  
 Fig. 2. Dwarf form of *danica*, from normal *danica* selfed. (Photo natural size.)  
 Fig. 3. Plant with folded type of leaf,  $F_2$  from *officinalis*  $\times$  *danica*.

## PLATE VII.

- Fig. 4. *C. officinalis*, *danica*, *anglica*, *officinalis*  $\times$  *anglica*, *anglica*  $\times$  *officinalis*, and *officinalis*  $\times$  *danica*,  $F_1$  plants.

## PLATE VIII.

- Fig. 5. Plants of Fam. 32/22,  $F_2$  from *danica*  $\times$  *officinalis*. Numbers 1 and 2 are two of the largest, and Number 7 one of the smallest plants in the family.

The seeds which gave the plants shown in Plates II and III were sown on the same day. The photographs were also taken on the same day.

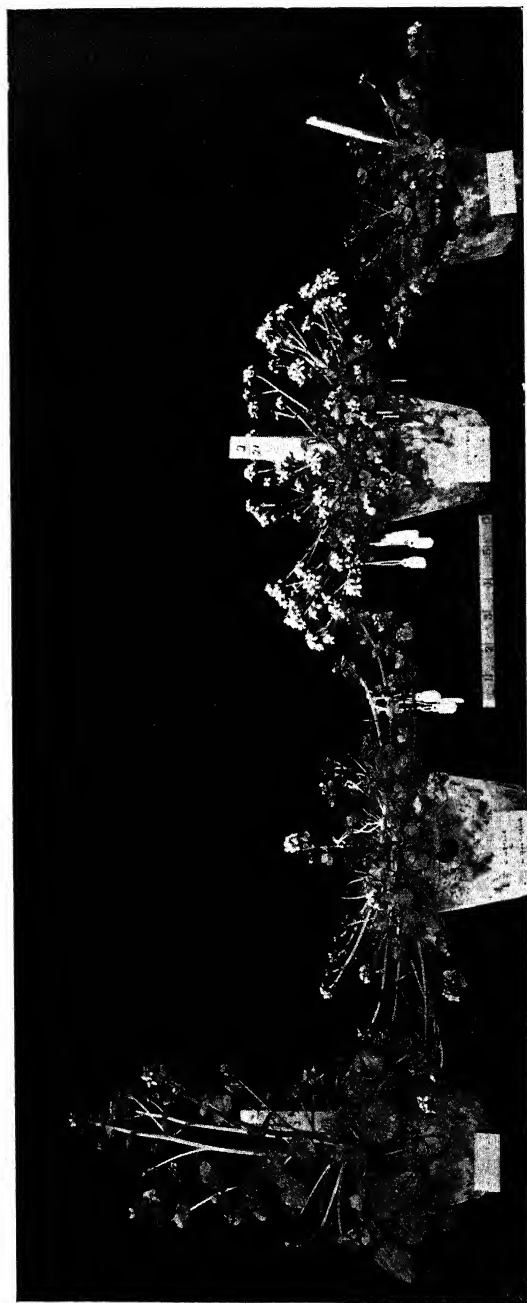
## PLATE IX.

All figures drawn with the aid of a Leitz camera lucida, with 1/12 oil immersion objective and 18 eye-piece, magnification 2250. All equatorial plates in root-tips.

- Fig. 1. *C. officinalis*. 28 chromosomes.  
 Fig. 2. *C. danica*. 42 chromosomes.  
 Fig. 3. *C. anglica*. 49—50 chromosomes.  
 Fig. 4. *C. anglica*  $\times$  *C. officinalis*. 39 chromosomes.  
 Fig. 5. Plant 5/21. 24—26 chromosomes.  
 Fig. 6. *C. alpina*. 28 chromosomes.  
 Fig. 7. *C. officinalis*  $\times$  *danica*, plant 15/21. 35 chromosomes.  
 Fig. 8.  $F_2$ , *C. danica*  $\times$  *officinalis*, plant 30<sup>1</sup>/22. 28 chromosomes.  
 Fig. 9. *C. danica*  $\times$  *officinalis*, plant 25/21. 35 chromosomes.  
 Fig. 10.  $F_2$ , *C. danica*  $\times$  *officinalis*, plant 30<sup>7</sup>/22. 35—36 chromosomes.  
 Fig. 11.  $F_2$ , *C. danica*  $\times$  *officinalis*, plant 30<sup>11</sup>/22. 38 chromosomes.  
 Fig. 12.  $F_2$ , *C. danica*  $\times$  *officinalis*, plant 30<sup>4</sup>/22. 39—40 chromosomes.  
 Fig. 13.  $F_2$ , *C. danica*  $\times$  *officinalis*, plant 32<sup>8</sup>/22. 38 chromosomes.  
 Fig. 14.  $B \times 1^1/21$ , plant 25<sup>9</sup>/22. 38—40 chromosomes.  
 Fig. 15.  $1^2/21 \times B$ , plant 19<sup>1</sup>/22. 35 chromosomes.

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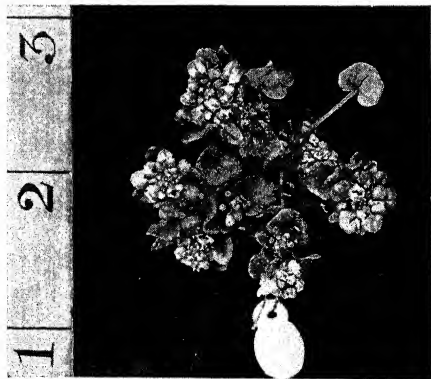


*C. officinalis.*

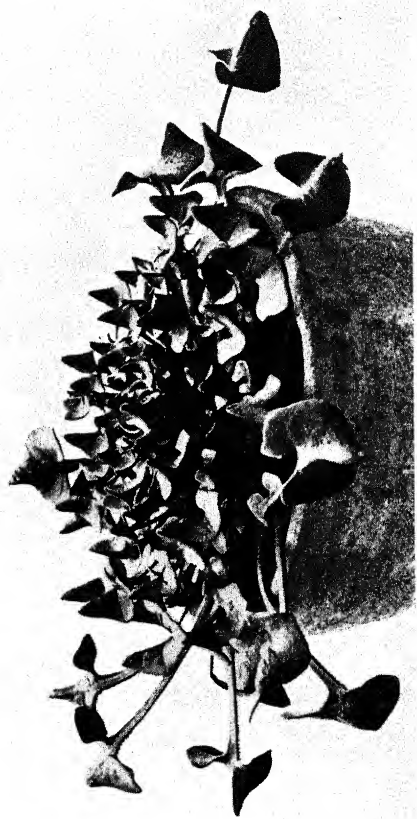
*C. danica.*

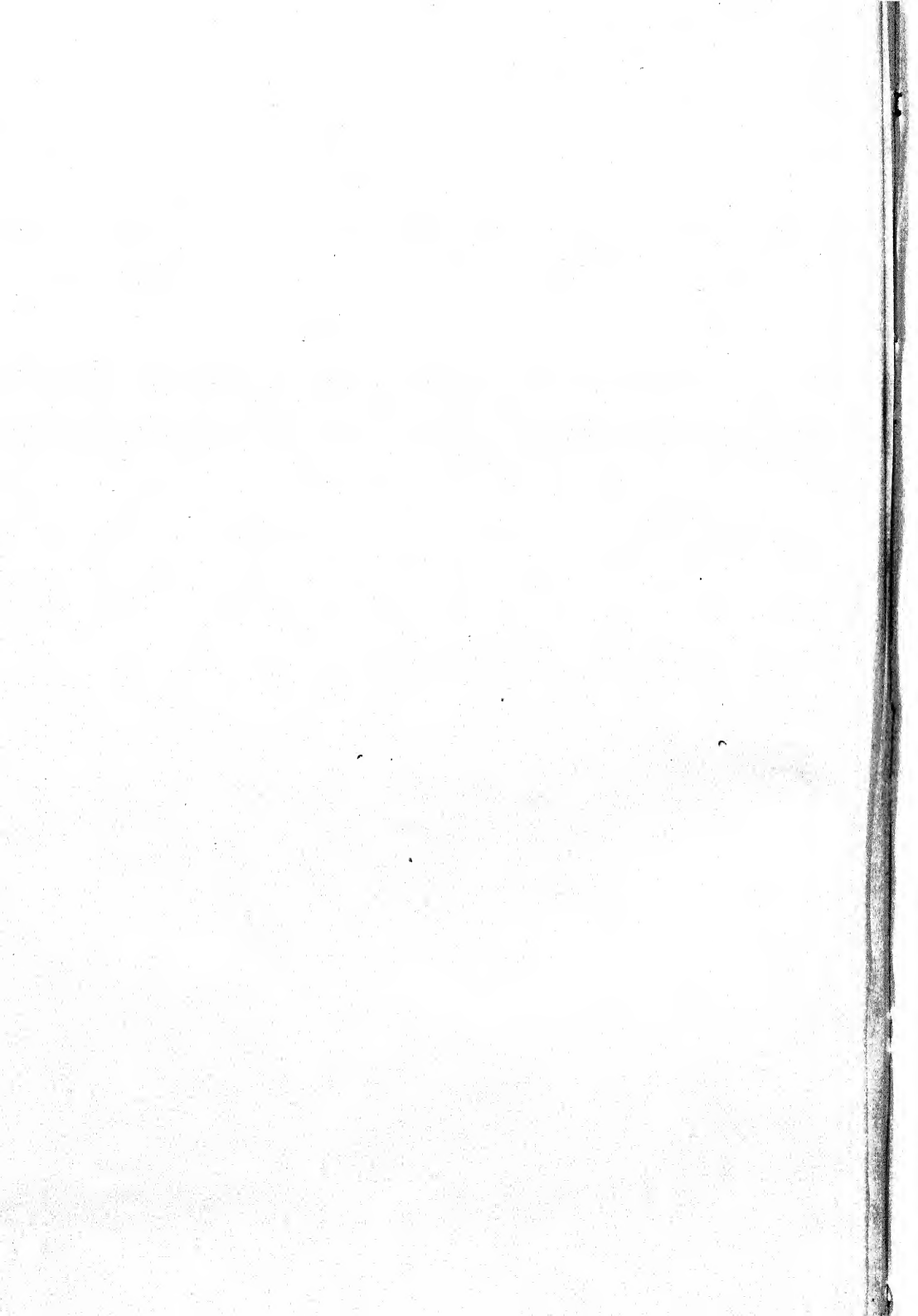
$F_1$

Fig. 1.



1 2 3







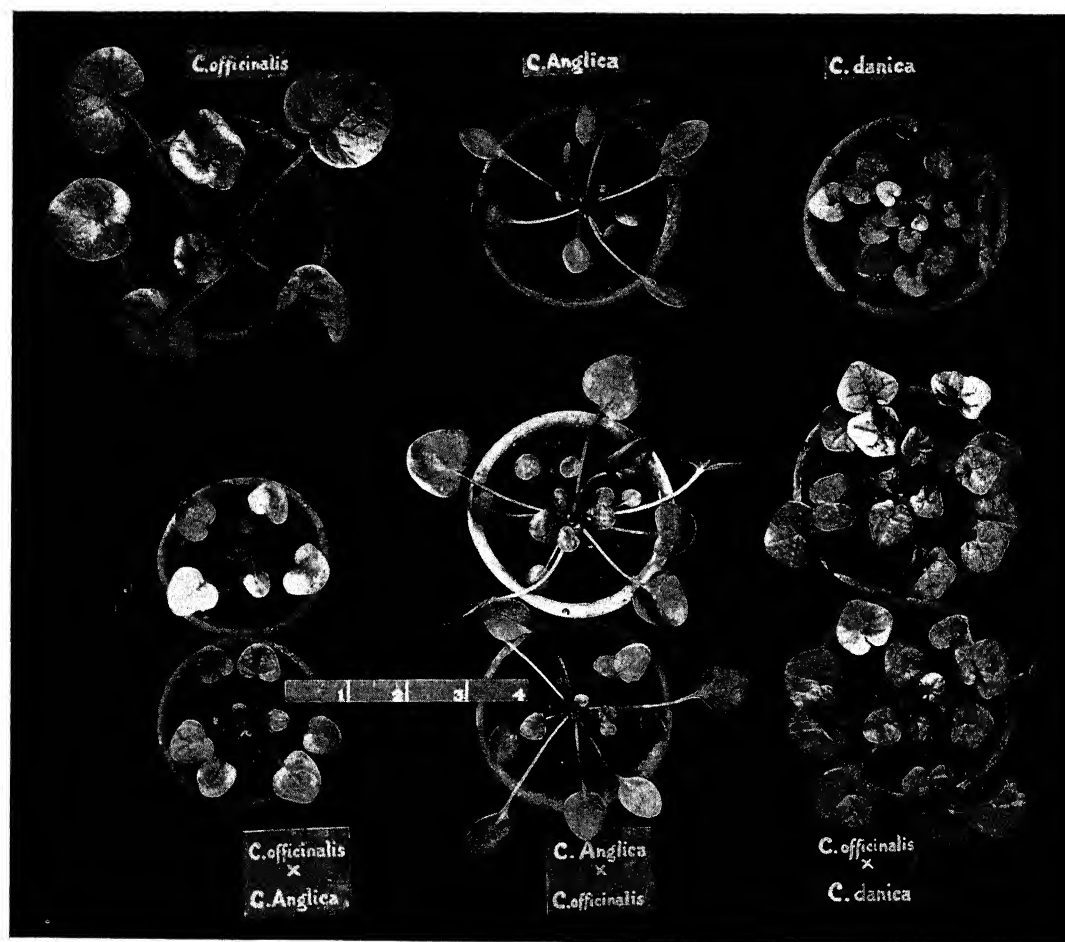


Fig. 4.





Fig. 5. Fam. 32/22, *F*<sub>2</sub> from *danica* × *officinalis*.





1



2



3



4



5



6



7



8



9



10



11



12



13



14



15



## CROSSING-OVER BETWEEN THE X- AND THE Y-CHROMOSOME IN *LEBISTES*.

By Ö. WINGE.

(Report from the Genetic Laboratory of the Royal Veterinary  
and Agricultural College, Copenhagen.)

(With One Text-figure.)

IN a paper on inheritance in *Lebistes reticulatus* (1922 b), discussing sex-linked as well as one-sided male inheritance, I mentioned an observation of crossing-over between the X- and the Y-chromosome in the male *Lebistes* and stated that further details would be published later. It is the object of the present report to fulfil this promise.

In the work of Schmidt on *Lebistes* (1920), the first unquestionable case was brought forward on conditions of inheritance which pointed to factors connected with the Y-chromosome. The investigations made by me, at a later date, on the cytological conditions in *Lebistes* (1922 a), have shown that the diploid chromosome number was 46 in both sexes—and in my first-named paper I stated, as the most important result of my experimental cross-breeding that, notwithstanding the lack of any visible difference between the chromosomes themselves, the male *Lebistes* decidedly belonged to the XY-type and the female to the XX-type, as is the case with *Drosophila melanogaster*. From the one-sided male inheritance, which could only be explained by assuming the existence of a factor-bearing Y-chromosome, one must logically conclude that X-chromosomes were also present and, thus, that the expression for the female chromosomes was  $44 + X + X$ , that of the male one  $44 + X + Y$ .

Moreover, I was able to prove sex-linked inheritance in at least one of the *Lebistes*-“races” which I worked on, a further instance of the indubitable conformity between the results of genetical and cytological investigations.

At the time I communicated the first case of crossing-over between the X- and Y-chromosomes,—a phenomenon which had not hitherto been brought to light for the simple reason that no factor-bearing

Y- (or W-) chromosome had hitherto been known with certainty to exist—I had no idea that another person was studying the same matter and had simultaneously arrived at the same results as I had.

The Japanese scientist, Aida, has undertaken extensive experimental crossings with another species of fish, the *Aplocheilus* (*Haplochilus*) *latipes*, belonging to the Poeciliidae family, published in *Genetics*, Vol. VI. 1921, p. 554<sup>1</sup>. In this paper, he not only proves the one-sided male inheritance, but also the crossing-over between the X- and the Y-chromosome, so that our two observations must be said to cover each other. However, no cytological investigation of the material worked on by Aida has been published as yet.

As in my own case, Aida has studied colour-factors which he found to be present in the autosomes as well as in the sex-chromosomes. In one case he discovered homozygosity for a factor *R* (red), i.e. the "race" concerned carries the *R*-factor in the X- as well as in the Y-chromosome. In cross-over individuals segregated from heterozygotic males, the conditions of inheritance are exactly as would be expected on the supposition that the factor in question (*R*) had been transferred from the X- to the Y-chromosome.

While, in *Lebistes*, the colour-factors manifest themselves in male individuals only, the inheritance being phenotypically sex-limited to the male—a fact which has not made my researches any easier—the colour-factors appear in both sexes of *Aplocheilus*. There is another slight divergency in our investigations, viz. while the *Lebistes*-factors influence patterns and various colours in certain parts of male individuals, the *Aplocheilus*-factors involve a, so to speak, total differentiation of colour in the individuals.

Hitherto, only the *R*-factor (allelomorph *r*) has been found in the X- and Y-chromosomes of *Aplocheilus*; while in *Lebistes* the Y-chromosome has been proved to contain a whole series of factors, and the X-chromosome, until now, only one factor, *s*, which has been seen only in one instance to cross over, *partially*, to the Y-chromosome, the said factor *s* being a complex factor composed of a series of linked factors. On the other hand, no colour-factors have, as yet, been found in the autosomes of *Lebistes*, whereas Aida has recorded the factor *B* and its allelomorphs *B*<sub>1</sub> and *b*.

In neglecting these minor differences, the results concerning the two species of fish must be said to confirm each other.

<sup>1</sup> This part of *Genetics* appeared Nov. 1922, though bearing date Nov. 1921.



As to the genetical formulae, Aida and myself use unlike expressions to which I shall revert below, in connection with the presence-absence theory.

I shall now proceed to the relation of my experiments on the crossing-over between  $X$ - and  $Y$ -chromosomes.

*Experiments on X-Y Crossing-over.*

In June 1917, while I was attached to the Carlsberg Laboratory, we received a male *Lebistes* which, in the main, had the appearance of the afore-mentioned (1922 *b*) "Spot Race" of which the males have the formula  $X_oY_m$ . The fish was bought from a Mr Christensen, Poppelgade (= Poplarstreet), Copenhagen, who, in his turn, had received it from Berlin, in the summer 1916, from a dealer in aquaria.

This new type had the following characteristics in common with "Spot Race" males, viz. (1) large black spot on dorsal fin; (2) large red side-patch below and in front of dorsal fin; (3) black dot near anal aperture; all being due to the *maculatus*-factor  $m$  in the male  $Y$ -chromosome. But whereas all the "Spot Race" males I had hitherto seen possessed a rounded, colourless caudal fin, the above male had (4) a caudal fin with elongated upper edge and (5) vivid reddish-yellow colouring on upper and lower edge of caudal fin.

This "Poplarstreet" male No. 123, as it was named after the place where it was bought, was mated, on June 9, 1917, with a virgin female No. 125 without factors in the  $X$ -chromosomes and, therefore, corresponding to formula  $X_oX_o$ , whereupon the female bore, until Dec. 27, 1917, six broods numbering more than 200 young ones in all. 73 young males became adult, but only one of these had the elongated caudal fin of the "Poplarstreet" male, all the others having the rounded colourless caudal fin of the "Spot Race."

Accordingly, the factor  $e$  (*elongatus*), responsible for the elongated caudal fin, must be supposed to reside in the male  $X$ -chromosome, which we know to pass on to the daughters; and we may also surmise that the single male with an elongated caudal fin owes its existence to the crossing-over, in a single case, of the  $e$ -factor to the  $Y$ -chromosome of a spermatocyte. The original "Poplarstreet" male having thus the formula  $X_eX_m$ , and therefore usually forming sexual cells carrying either  $X_e$  or  $X_m$ , it must have happened at the crossing-over that, in an isolated case, the  $e$ -factor was transferred to the  $Y$ -chromosome and then linked to the  $m$ -factor there. The formula of the diverging male should therefore be  $X_oY_{em}$ .

The inference, that the crossing-over between the  $X$ - and the  $Y$ -chromosome has really taken place here, is fully confirmed by the following crossing-experiments undertaken as a check. The process of analysis is represented in Fig. 1, under the form of a genealogical diagram.

As far as the non-cross-over individuals are concerned, the  $F_1$ -females of this category are supposed to have the formula  $X_e X_e$  and, in mating these females with  $F_1$ -males, the formula of which is  $X_o Y_m$ , the outcome should be an equal number of males with elongated caudal fin, and without. In accordance with this,  $F_2$  gave 26  $X_e Y_m$  and 27  $X_o Y_m$ . The  $F_2$ -females were also expected to consist of two categories, partly  $X_o X_o$ , partly  $X_e X_o$ , and in fact, one  $F_2$ -female, mated with a "Spot Race" male ( $X_o Y_m$ ), produced only  $X_o Y_m$ -males (11 in all) and therefore had the formula  $X_o X_o$ , while another  $F_2$ -female, mated with the same male, gave 4 males with elongated caudal fin ( $X_e Y_m$ ) and 3 without ( $X_o Y_m$ ); consequently, this latter  $F_2$ -female must have had the formula  $X_e X_o$ .

As stated above, the non-cross-over males should, theoretically, have the formula  $X_o Y_m$ , and their structure entirely corresponded thereto, i.e. rounded caudal fin and the general appearance of the "Spot Race." As a matter of fact, some such  $F_1$ -males, crossed with  $X_o X_o$ -females, produced exclusively round-tailed progeny. 22 sons in all were counted and none of these showed any trace of the  $e$ -factor.

It has thus been proved that the elongated caudal fin of the "Poplar-street" race is due to a factor in the male  $X$ -chromosome and that, therefore, the original male must have had the formula  $X_e Y_m$ . While the inheritance of factor  $m$  is one-sided male, the  $e$ -factor is sex-linked in its inheritance.

We shall now examine the conditions of the diverging  $F_1$ -male with the elongated caudal fin, so as to find out whether its genetical behaviour corresponds to the hypothesis that the  $e$ -factor has crossed over to the  $Y$ -chromosome, making the formula of the individual =  $X_o Y_{em}$ .

The male progeny of such a male should, of course, always be provided with an elongated tail-fin, all the sons receiving the  $Y$ -chromosome. Only in case of a renewed crossing-over could the  $e$ -factor again be separated from the  $m$ -factor and round-tailed sons be segregated.

All the 11 sons of the diverging male proved to have an elongated caudal fin, and this is already a proof that the  $e$ -factor has no longer its seat in the  $X$ -chromosome, but in  $Y$  and that, therefore, a crossing-over between  $X$  and  $Y$  must have taken place.

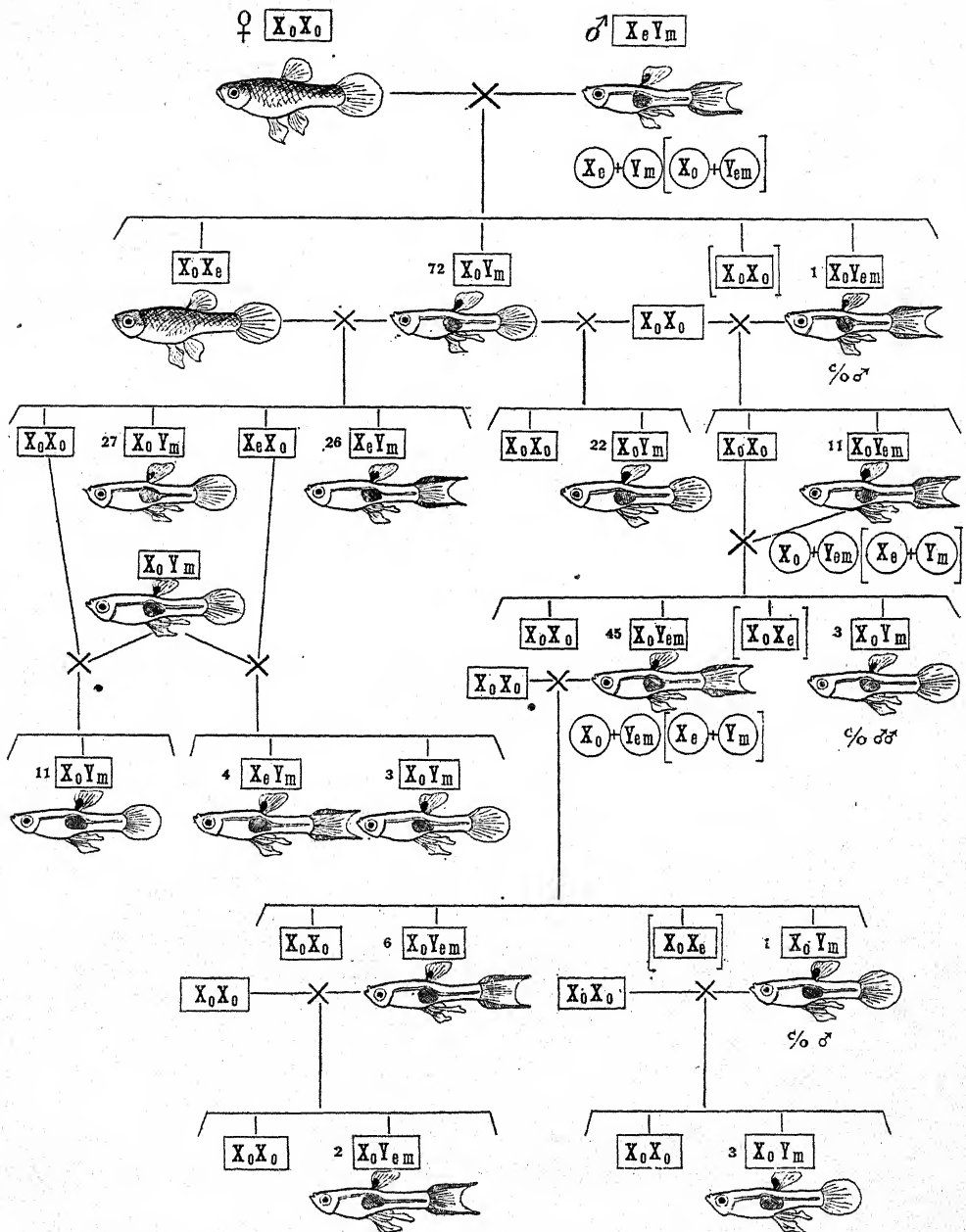


Fig. 1.

The sequel of the analysis gave the following result: The 11  $X_o Y_m$ -males were mated with some sisters which should correspond to formula  $X_o X_o$ , thus giving rise to a new generation,  $F_2$ , of cross-over males. As to the males, this generation consisted of 45  $X_o Y_{em}$  and 3  $X_o Y_m$ . In this connection, the 45 young ones are non-cross-over individuals, whereas the 3 are the result of a new crossing-over in which the  $Y$ -chromosome has again lost its  $e$ -factor, the latter having crossed back to the  $X$ -chromosome, its original position.

Among the offspring of the 45  $X_o Y_{em}$ -males, as shown in Fig. 1, was again found 1 round-tailed  $X_o Y_m$ -male, the result of a new crossing-over. As might be expected, this male, when mated with an  $X_o X_o$ -female, reproduced exclusively its own type, while the other individuals of this and the following generation all have the elongated caudal fin and the formula  $X_o Y_{em}$ .

Cross-over females are of course not so easily discerned as are cross-over males, as the females phenotypically show no colours at all. The only way in which we can recognize the factor-contents of a female individual is through crossing it with a male of well-known genetic constitution.

In a single case I have found such a primary cross-over female. All the female progeny from a cross-over male,  $X_o Y_{em}$ , with a female,  $X_o X_o$ , will be  $X_o X_o$  if no crossing-over between  $X$  and  $Y$  in the male happens. An  $X_o X_e$  female only can arise through crossing-over. Among the  $F_2$  offspring of the primary cross-over male,  $X_o Y_{em}$ , with an  $X_o X_o$  female, 6 female individuals were analyzed by pairing them with  $X_o Y_m$  males (Spot Race). The five bore only  $X_o Y_m$  males, 32 in all, and were therefore ordinary  $X_o X_o$  females, but No. 6 has until now borne 2  $X_e Y_m$  males and 3  $X_o Y_m$  and must therefore have the formula  $X_o X_e$ , i.e. be a female individual arisen through crossing-over in an  $X_o Y_{em}$  male.

We have thus proved that the  $e$ -factor, accountable for the elongated, coloured caudal fin of the original male individual, had its seat in the  $X$ -chromosome and was, therefore, sex-linked in inheritance ( $X_e Y_m$ ). By crossing-over between the  $X$ - and the  $Y$ -chromosome, it was, in one case out of 73, transferred to the  $Y$ -chromosome ( $X_o Y_{em}$ ), whereupon the inheritance of the elongated caudal fin became subsequently one-sided male. In the 4 male individuals out of 68, the  $Y$ -chromosome had again lost its  $e$ -factor which had anew crossed back to the  $X$ -chromosome, where it was found in a female individual.

An individual belonging, in all evidence, to the above-mentioned "Poplarstreet Race," was bought in Sept. 1917, from another Copen-

hagen dealer in aquaria and was said to have arrived from Dresden a month earlier. The race was, in all respects, similar to the "Poplarstreet Race" and, after a cross with a "Spot Race" female  $X_oX_o$ , offspring resulted, of which 22 were males, all with a rounded, colourless tail, showing that, in this individual also, the  $e$ -factor had its seat in the  $X$ -chromosome and that this male, therefore, also had the formula  $X_eY_m$ .

The whole matter will now appear clear and we shall therefore discuss the deductions which may be made.

#### *Theoretical Considerations.*

In the present and in a former report, I have shown that crossing-over between the  $X$ - and the  $Y$ -chromosome takes place in *Lebistes* and that the sole real difference between their  $X$ - and  $Y$ -chromosomes is that the  $Y$ -chromosome contains a dominant male factor, while the  $X$ -chromosome contains a recessive female factor. The  $e$ -factor, which produces the elongated, coloured caudal fin, may cross over from  $X$  to  $Y$  and vice versa, which, in other words, means that the male and the female factor are evidently allelomorphs which, like other factors, may be exchanged between the chromosomes. Nor has a cytological examination revealed any morphological difference between the  $X$ - and the  $Y$ -chromosomes.

Therefore, if it be true that the  $Y$ -chromosome contains a special male factor, this factor must be rather strongly linked to the  $e$ -factor, for only one cross-over individual has been found among the 73 sons of an  $X_eY_m$ -male and 4 cross-over individuals among 68 sons of  $X_oX_{em}$ -males.

It is remarkable that crossing-over has not been equally frequent in both directions; however, as the amount of material was not very large, I do not feel justified in attaching any significance to this fact. Much stronger is, however, the linkage between the male factor and other factors found in the  $Y$ -chromosome, such as  $m$  (*maculatus*),  $i$  (*iridescens*), etc. and the circumstance itself of diversity in the degree of linkage between sex- and colour-factors must be taken as evidence of a real gene, corresponding to the sex-factor, embodied in the chromosome and comparable with other genes. As to the  $m$ -factor, thousands of individuals, produced after mating of  $X_oX_o$ -females with  $X_oY_m$ -males, have been examined without one single individual with the  $m$ -factor missing being found. This is very striking. At the outset, one sees no reason why these factors should be located so close to the male factor that no crossing-

over can take place, but, as stated above, it is quite possible that the male factor itself is identical with the *m*-factor, although in that case one must arrive at the conclusion that, with regard to sex-factors, multiple allelomorphs exist. Another explanation of the strong linkage of these colour-factors to the sex-factor would be that crossing-over is rather rare in *Lebistes*. As said in my earlier paper (1922 *a*), I have looked in vain for a typical stage of synapsis in the spermatocytes, and neither has diakinesis been observed. Perhaps the explanation of the strong linkage might also be that only factors situated at some distance from each other can be exchanged, on account of a pronounced rigidity of the chromosomes.

It is certain that great difficulties prevent a close study of the topography of the *Y*-chromosome; for on one hand, the linkage between the colour-factors and the sex-factor appears to be strong, while, on the other hand, the factor-contents of the female individuals cannot be directly ascertained. The future must decide whether factors will be found which are linked to the sex-factors in a sufficiently variable degree to make charting of the sex-chromosomes feasible. It is obvious that, in any case, as far as *Lebistes reticulatus* is concerned, many colour-factors exist which have not yet been analysed, for, although the variations of appearance within one single race are limited, a considerable number of *Lebistes*-types are found with dealers in aquaria as well as wild.

I have mentioned that, in several of the *Lebistes*-races examined, the *X*-chromosome is empty, e.g. in the "Spot Race," and have therefore been given the sign  $X_0$ . In this case, as well as in all other *Lebistes*-races observed, the *Y*-chromosome alone contains a colour-factor. In comparing this circumstance with the fact that the *Y*-chromosome of *Drosophila* is empty, the comparison is not entirely to the point, in as far as I have reckoned with the existence of a female factor in the *X*-chromosome of *Lebistes*, whereas the existence of a corresponding male factor in the *Y*-chromosome of *Drosophila* has not been proved. On the contrary, according to the latest, very interesting researches of Bridges (1922), on flies with an abnormal set of chromosomes, it seems as though the ratio between the numbers of autosomes and of *X*-chromosomes alone determines the sex in *Drosophila*.

At any rate, factors in *Lebistes* have been found, as yet, only in the sex-chromosomes, while not one has been discovered in the 22 autosomes. Notwithstanding this, the autosomes are undoubtedly factor-bearing, even if no special colour-factor exist in them. We do not know yet in how far species determining chromatin is present in the chromo-

somes and whether it is common to all the chromosomes of one species. As only fundamental *differences* can be made the object of inheritance researches, nothing hinders the supposition that a great many species factors exist homozygotically in all the biotypes of the species, and it is even probable that several chromosome-pairs contain, in principle, the same specific factors. This latter hypothesis seems to be confirmed to some extent by the fact that numerical progressions in the number of chromosomes are found within systematic genera of the vegetable kingdom, e.g. 7, 14, 21 (*Triticum*) or 9, 18, 27, 36, 45 (*Chrysanthemum*), etc., which progressions can only be explained by supposing that the higher numbers are the result of the addition of two lower chromosome numbers after hybridization—or the doubling of the original chromosome-set. New polyploid species, originated by hybridizing two earlier species, with a simultaneous doubling of the chromosomes,—the probability of which I have established in previous researches (1917)—will have the genus-fixing factors redoubled, but this feature is not traceable in the descendancy of the new types, because they are homozygotic in the genus-fixing factors, or even several times homozygotic. The fact that polymeric factors frequently occur in polyploid species—well known for instance from Nilsson-Ehle's studies of *Avena sativa* and *Triticum vulgare*—fully agrees with the supposition that the factors are repeated several times in the polyploid species; and it is quite natural to assume that even species which, on account of their number of chromosomes, do not reveal themselves as polyploid, contain many specific generic factors which are common to all the chromosomes. Some chromosome researches seem to show that adhesion between originally freely "mendelizing" chromosomes may take place; and a chronic conjugation of chromosomes, in connection with above conditions, will result in a gradual, manifold appearance of the same factors in each single chromosome—or, in any case, in more than one.

According to this, all genera of a given family should be characterized by the factors peculiar to that family, but they should be distinguished from each other by "genus-factors." All the species of a genus should contain identical factors, characteristic of that genus only, and furthermore a great many factors distinctive of the family, and be distinguished from each other only by a smaller number of specific species factors. All the varieties within one species should, homozygotically, contain identical "species-factors" beside the particular genus- and family-factors which will probably be present in several or in all the chromosomes—and they should be mutually distinguished by one or a few "variety-



factors" having their seat in one or in a few chromosomes, and disclosing themselves in Mendelian segregation when the varieties are crossed.

As two varieties, chosen within a species, only differ as far as a few factors, the "variety-factors," are concerned, but are homozygotic and isogenous with regard to all the species-, genus-, family-, etc. factors, the effect of variety-factors is alone observed in segregations. The larger the systematic unit under consideration, the more often must the genes, characteristic of that unit, be repeated in all the chromosomes.

Of course, one could not think of any absolute boundary between, for instance, "species-factors" and "variety-factors," but we must suppose that, in substance, those factors which are widely represented in all the chromosomes afford a basis for classification into large, systematic units; their frequent presence in the chromosomes will entirely prevent a segregation into types in which these factors are lacking and, with them, the corresponding morphological and physiological particularities; and the loss or the modification of a few such factors could hardly provoke any immediately visible mutation, because many others of the same nature would be left. At the most, this eventuality could be imagined to tend, throughout a long period of time, towards a slow shift in the characters of the species—a condition of things which might be associated with biological evolution.

#### *The Presence-Absence Theory. The Denomination of Factors.*

According to the opinion of many geneticists, on the nature of factors, the type holding the dominant factor contains a gene which is missing in the type holding the recessive factor. This theory has been named the "Presence-Absence Theory." It can be said to have given satisfaction until multiple allelomorphs were recorded, but after that it fell short, as the existence of a certain number of *different* types, all recessive to one and the same dominant type, could of course not be explained by the absence of the gene corresponding to the dominant factor. The notion imposed itself that, like the dominant type, the recessive type possesses a gene, but that this gene differs in value from that of the dominant type—and has a different value in the different recessive allelomorphs. According to this, multiple allelomorphs should originate from genes, diverse in value, but having the same location in the chromosomes. This conception is unquestionably gaining ground.

It has been seen from my *Lebistes*-formulae that I use the denomination  $X_0$  for an  $X$ -chromosome which contains no colour-factors, i.e. a



mode of denomination in conformity with the original conception,—and this has been done deliberately.

Aida applies the formula  $R_x r_y$  to a male which I would designate by  $X_r Y_o$ . In the first place, Aida uses  $X$  and  $Y$  as indexes showing the location of the factors considered and thereby gives more prominence to said factors. In my own work, I have made a rule of using the factors as indexes to the chromosome-denominations, for the reason that, to keep abreast with the recording of more and more factors in each chromosome, an expression as, for instance,  $AA X_{rspn} Y_{rv}$  admits, according to my idea, of more rapid discrimination than  $AA R_x R_y S_x P_x N_x V_y$ . With my system, the factors and their location in each of the chromosomes are seen at a glance. As the charting of autosomes progresses, their factors could be represented in the same manner, e.g.  $I_{thl} II_{bcd}$ , etc. and the factor-set of a diploid type by  $I_{tthll} II_{bbccdd}$ , this latter example corresponding to a homozygotic individual. In consequence, I would first of all change Aida's denomination,  $R_x r_y$ , into  $X_R Y_r$ .

Furthermore, Aida has chosen  $R$  to represent the factor for the red colour, as most geneticists would have done, and  $r$  for the recessive allelomorph of this factor, whereas it would have been more descriptive to denote the factor for red by  $r$  (or  $R$ ) and its absence by  $O$ ; whether  $R$  or  $r$  should be chosen, would depend on whether red is dominant or recessive in relation to the normal type. My reason for choosing  $O$  to denote the absence of the colour-factors is that precisely the study of *Lebistes* (and *Aplocheilus*) very decidedly tend to bring about the conception that, whichever be the colour-factor considered, its allelomorph will be equal to  $O$ , i.e. that the corresponding gene is missing in the "recessive" type or is, in any case, inactive.

I have shown that, as crossing-over takes place between them, the  $X$ - and the  $Y$ -chromosomes are partners to the same extent as the other chromosomes in *Lebistes*. According to the current notion, each factor in the  $Y$ -chromosome should therefore have a corresponding allelomorph in the  $X$ -chromosome, and vice versa. Thus, the factors which I have called  $m$ ,  $i$ ,  $f$ ,  $r$ ,  $s$  and  $e$  should all have their corresponding allelomorph factors in the chromosome partner; but we have seen in the crossing experiments that whether we take a female in which  $m$  is lacking, or one in which  $i$  is lacking—i.e. whether the female belongs to the "Spot Race" or to the "Old Race"—it contains the same factors, that is to say none ( $X_o X_o$ ) from the accountant's point of view, as far as colour-factors are concerned, or, in other words, only inactive or neutral genes. According to the current mode of denomination, my *Lebistes* females

should, in one case, have the formula  $X_m X_m$ , i.e. those of the "Spot Race," where the males should be called  $X_m Y_m$ ; but if belonging to the "Old Race," their formula would be  $X_i X_i$  and that of the males  $X_i Y_i$ ; but whether we use the females of one race or the other, the result is exactly the same: the two kinds of females are identical as to inheritance, i.e. they are  $X_{mi} X_{mi}$ , a formula which is in so far irrational as  $m = i$  and equal to all the other "recessive" allelomorphs, which are all equal to  $O$ .

This discussion touches the question of the right conception and denomination of genes or factors, and much would undoubtedly be gained if a uniform mode could be agreed upon.

In *Drosophila*, the organism which has hitherto been the most extensively studied, the "Normal Type," i.e. the wild type, is the established base upon which the whole terminology of factors has been built up. When forms appear, which are recessive with regard to this base, they are denoted by the corresponding small letters, while new dominant forms are denoted by capitals.

In *Antirrhinum*, there is no such decidedly normal type to refer to, and we might say that, here, the denomination of factors generally refers to an entirely recessive type.

In reality, the results are alike. In both organisms, the value of the factors is established in relation to a given type of fixed habitus or—in case the entirely recessive type is not viable—in relation to a type, the aggregate characters of which have been determined theoretically.

It makes no difference whether the type in question is measured or characterized in comparison with a wild type, chosen as "normal," or with an entirely recessive type. In both cases, the notation is relative, because it implies reference to a type, an individual of the same species, with which all other types are then compared.

Genetical science does not seem to take any interest in the systematic basis of the species as such. But we should say that this is an appearance only.

The present system of denoting factors—or rather pairs of factors—unavoidably presupposes that all types belonging to a species possess the same number of genes: any type must either possess the recessive or the dominant allelomorph of any pair of factors.

On reconsideration, this is a rather curious idea, which immediately calls forth the question as to whether all the species of a genus, all the genera of a family, etc. also have the same number of genes. Logically, and from a formal point of view, this hypothesis need not yet run against any self-contradiction. Taking *Antirrhinum* as an instance, and  $F$  to

stand for the red of its flowers, there could be no objection to the use of *ff*, not only for all ivory-coloured *Antirrhinum*, but also for all white peas or all animal organisms; for, in fact, none of the latter possess the predisposition to red of the *Antirrhinum* corolla. This, precisely, shows that, in the study of inheritance, the tendency to formalism is becoming rather pronounced.

There is hardly any reason whatever to suppose that two different varieties possess an equal number of inheritance-elements, i.e. genes. Even as the different species of a genus may hold chromosomes of entirely differing number and aspect, and therefore—if our conception of the genes, as arranged along the longitudinal axis of the chromosomes, is to hold at all—must have a widely differing number of genes, so it must no doubt also apply to the varieties within the species, that they need not contain the same number of genes.

The mistake we make in continually considering the number of genes as constant and identical in all related (and unrelated) organisms, very likely derives from the fact that our conception of the allelomorphs is somewhat erroneous and that, in principle, we must revert to the old presence-absence theory which, alone, explains why a type, a variety, a species, etc. can hold certain genes that are entirely lacking in others, where not even a corresponding gene-partner is found.

Not only in this connection do we get the impression that the presence-absence theory holds good. This applies to almost any genetic question. Of course, it makes no great difference whether, to explain a recessive factor, we assume the existence of a gene which is, under all circumstances, *inactive*, or whether we simply deny the existence of any such gene. But I suppose that the distinction between non-differentiating and differentiating elements really ought to find its expression by attributing the denominating "genes" exclusively to the latter, while the former should, from a genetical point of view, be represented by zero (0), as having no traceable influence on the sum of inherited elements of the organism. In reality, it is perhaps unessential whether the factor-denomination is assigned to the dominant or to the recessive allelomorph (its partner being put equal to 0). At any rate, it will not always be possible to decide which one of the two allelomorphs should have the factor index. It seems natural, however, as all recessive allelomorphs, as *m*, *i*, *f*, *r*, etc., have the same indeterminate value, to give them the value zero; in fact there is no difference between them, neither relatively nor absolutely. Although the study of inheritance essentially concerns relativity and fundamental *differences*, the real and, to my

mind, indispensable basis, would be generally to take 0 as the value of the recessive factors or genes and to count with positive values of recessive factors or genes only in such more rare cases as, for example, multiple allelomorphs. Referring to the latter case, one could imagine some slight shift in the chemical nature of the gene—provoking a special activity of each of the allelomorphic genes—to be the cause of the appearance of multiple allelomorphs; and in the first case, a shift in the chemical nature of the gene, to such an extent that the gene entirely lost its differentiating influence on the type.

In spite of this opinion, I have no doubt but that it would generally be inconvenient to apply another mode of denomination to the factors, than the present one. Zero as an index for the lack of a factor, would often be less suitable than a small letter, partly, because one is not always able to decide whether an allelomorphic factor is really missing in the case under consideration, partly, because the use of a small letter would often give a clearer formula. On the other hand, it would be convenient, when comparing to a "normal type," to be able to symbolize the factor, characterizing a different and recessive type, by a small letter, for instance *a*, the allelomorph of the normal type being here equal to 0, and to symbolize the factor of a dominant type by a capital letter, say, *B*, the allelomorph of the normal type being again 0.

Although, for my work on *Lebistes*, I have tentatively used a mode of denomination which was, in my opinion, more correct than the one used hitherto, I am quite aware that this method might cause some inconvenience in certain cases. The future must decide upon the system to be preferred.

As said before, there can hardly be any question of dominance or recessivity as far as the colour-factors in *Lebistes* are concerned. In the female, no colour-factor whatever can be traced; and in the male, when comparing to *Drosophila* conditions, even recessive factors would evidently appear and, *a fortiori*, the dominant ones. As however, at variance with *Drosophila*, crossing-over between the X- and the Y-chromosomes is possible in *Lebistes*, we might say that the colour-factors dominate their recessive allelomorphs but, as the latter are equal to 0, the index for dominance does not mean very much in this connection, as in many others;—it would simply mean that the gene, if present at all, manifests itself phenotypically in the male.

Considering the sex-factors in *Lebistes*, it is possible that real allelomorph factors exist, i.e. the male and the female factors, although we might conceive that, here again, the male sex-factor alone acts positively,

whereas the female factor is 0, i.e. does not alter the female character in the male direction.

It will be one of the tasks of the future to determine the dimensions of the genes. By calculating the percentage of crossing-over in *Drosophila*, the location of a great many of its chromosome-factors has already been charted, taking it for granted that the cross-over percentage may still be considered mainly as some function of the distance separating said genes along the longitudinal axis of the chromosome. The dimensions of the chromosomes being known, as well as the number of factors recorded and the relative distance between these, one may vaguely conceive the smallness of the units of measurement which will have to be used in connection with the genes. Probably, the length of chromosome to be considered in such calculations should be that characteristic of the synapsis stage, or an adjoining stage; it is probable that the genes of the chromosomes we observe, for example during the heterotypical division, are not strung along a straight line, but bunched like the beads of a row, kept in a wide test tube or the like. Unfortunately, the length of the chromosome at the stage of synapsis cannot be easily measured; a rough estimate will, however, show that we are still far from dimensions as for instance those of the molecules of albumen.

Even from a slight crossing-over as, say, 0.2, between two strongly-linked genes, we can conclude nothing as to the size of the genes, for two genes may of course be in close vicinity, whether they are large or small. It is necessary to know at least three neighbouring genes before being able to estimate the dimensions of the space occupied by the middle gene,—and it would be important that more observations be made of such strongly-linked genes, for the furtherance of the knowledge of the size of the genes. By the way, we do not know whether the genes take up the entire length of the longitudinal axis of the chromosome.

#### *Sex-Linked, Sex-Limited and One-Sided Inheritance.*

As a contribution towards the adoption, in scientific literature, of uniform terminology for the various modes of inheritance (at present, such terms as "sex-limited" and "sex-linked" are often used at random), I would point out that the denomination "*sex-linked*" (*geschlechtsgebunden*) ought to be applied only to conditions of inheritance explainable by the presence of the factor in question in those sex-chromosomes which are normally found in individuals of both sexes (*X*- and *Z*-chromosomes). "*One-sided*" (*einseitig*) inheritance should be used

only in such cases where the factor in question has its seat in that sex-chromosome which is normally found in one sex alone (*Y*- and *W*-chromosomes), adding the mention *male* or *female* so as to specify whether said factor belongs to the *Y*- or to the *W*-chromosome. The value of this distinction is not lessened by the fact that crossing-over between *X*- and *Y*-chromosomes has been proved, for nothing opposes the notion that, through a genetic displacement, one mode of inheritance may change into another. As to the denomination "*sex-limited*" inheritance, we are less fortunate because the secondary sex-characters, to which the term is generally assigned, are usually due to autosome-factors which, however, produce an exclusively *phenotypical effect* in individuals of one of the sexes. The milking capacity in cattle, the production of lupuline in hop, are instances of so-called (female) sex-limited inheritance. In reality, it is not a question of any special mode of inheritance, so far as we know, but only of phenotypical differences dependent on sex-determination. For this reason, the expression "*sex-limited inheritance*" is not very judicious in any case. "*Sex-limited manifestation*" would be better. "*Phenotypically sex-limited inheritance*" might perhaps do.

#### RÉSUMÉ.

A factor *e* (*elongatus*), involving an elongated caudal fin in male individuals of *Lebistes reticulatus*, shows sex-linked inheritance, for which reason the *e*-factor must have its seat in the *X*-chromosome of the male.

Crossing-over between the *X*- and the *Y*-chromosome is recorded, the *e*-factor thereby being transferred from the *X*-chromosome to the *Y*-chromosome. Males having thus their *e*-factor in the *Y*-chromosome, show one-sided male inheritance of the elongated tail fin.

In these cross-over males, having the *e*-factor in the *Y*-chromosome, a new crossing-over may take place between *X* and *Y*, so that the *e*-factor returns to the *X*-chromosome, and so on. The mode of inheritance is therefore continually oscillating between sex-linked inheritance and one-sided male inheritance.

The above shows that the *X*- and the *Y*-chromosome in *Lebistes* respectively contain a dominant male sex-factor and a recessive female sex-factor; also that this pair of factors is entirely like other pairs because its location in the chromosome is certain, and because it shows linkage to colour-factors, crossing-over, etc. In the above respects,

the divergence from conditions in *Drosophila melanogaster* is rather striking.

The Presence-Absence Theory and an ideal terminology are discussed.

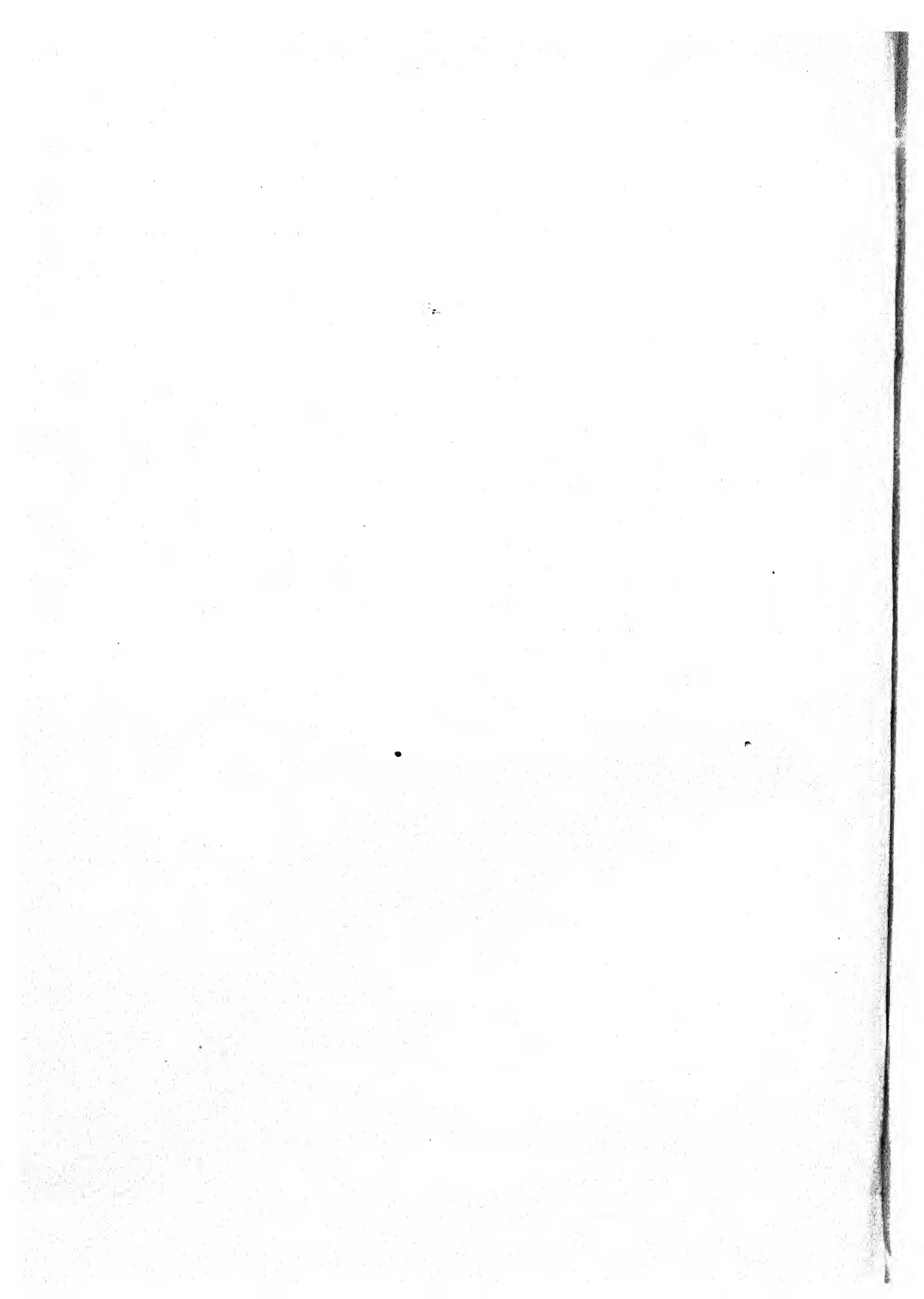
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## GENETICS OF *PRIMULA SINENSIS*.

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(With Plates X—XVII.)

AT the time of his death in 1918 R. P. Gregory was continuing the experiments on *Primula sinensis* which had occupied him since 1903. He published a paper in 1911 giving several results already arrived at, and subsequently notes dealing with special points. These publications however represent only a small part of the ground covered by his work, which in 1918 had already extended over many features not previously reported on at all. During his lifetime the plants were grown partly at the Cambridge Botanic Garden and partly at the John Innes Horticultural Institution. Those at Cambridge were, during the war, mainly in the care of Miss Killby, who carried out the manipulations. At Merton the work was undertaken by various members of the staff, especially Mr Backhouse, the late Mr E. J. Allard, Miss I. Sutton, Miss Thornton, also by Miss A. Sverdrup, and several other volunteers; but for some part of each year Gregory attended and recorded the results himself. After his death the Cambridge experiments were wound up, and the Merton section was somewhat extended. So much of the work we have now to describe owed its inception to Gregory, or has grown out of material with which he dealt, that his name most properly stands at the head of the present publication.

Some 18 pairs of characters have been investigated more or less fully. They relate to the forms of the leaf; the forms of the corolla; the anthocyanin colours and their distribution in vegetative structures, petals, and the gynoecium, especially the stigma; the extent of the yellow pigment surrounding the "eye" of the flower; the heterostyle condition; single and double flowers.

Interactions have been observed between factors affecting primarily the leaf-shapes, and those producing distinctions in the corolla-shape. Crimping of the leaf, for instance, is associated with fringing of the

petals, the tongue-shaped leaf with an elongation and narrowing of the petals<sup>1</sup>, etc. These are associations which might be expected, but the intensification of the crimping which results when the *sinensis* flower is transferred to the crimped leaf is curious. The most remarkable of these interactions is that between the factors governing the extent of the yellow eye and those affecting the margin of the leaf. This mass of interactions, involving three distinct sets of characters (petal shape, eye, leaf margin), has not yet been completely analysed. An outline of the facts is included in this paper. The essential points are

(1) The normal eye is not compatible with the crimped leaf which entails some extension of the eye.

(2) The most extended eye (that called "Primrose Queen") is compatible with a perfectly flat leaf; but when this eye is combined with a slightly crimped leaf, the crimping is intensified.

A good deal of information has been acquired as to the giant varieties, some of which are tetraploid, and as to defect of chlorophyll in the leaves, etc., but these two subjects are not treated of in the following paper.

#### MATERIAL.

The bulk of the plants were derived in part from those which had been experimentally bred in Cambridge since 1903, and in part from Messrs Sutton's collection. We acknowledge gratefully the constant assistance we have had from Messrs Sutton, who have put their experience unreservedly at our disposal. We have also to thank Messrs Carter for a set of their double-flowered oak-leaf variety which was brought into the work in 1916.

Besides these forms which are more or less familiar greenhouse objects, we received in 1913 a plant hereafter spoken of as "Lee's" which is new. Mr E. Lee, then of the Botanical Staff at the Birkbeck College, since killed in the war, called in 1913, bringing a plant with some novel characteristics. He said that this form had appeared spontaneously in the vinery of a private garden at Egham, and had since bred true. The edges of the palmatifid leaves were much *crimped* (details below). In correspondence with this peculiarity the petals were minutely crenelated. These crenelations were like those in the ordinary *sinensis* flower, but more numerous and more regular, giving the corolla a fringed appearance. The flowers were *double*, this doubling being of the peculiar kind called by Gregory (1911, p. 90) "ordinary" (i.e. for modern strains), in which

<sup>1</sup> The "oak" leaf causes a frequent deformation of *sinensis* flowers if single, but this effect is not noticeable in doubles or in the *stellata* forms (see Figs. 33 and 34).

each petal bears an extra limb arising at the mouth of the tube, standing in the reversed attitude characteristic of this type of doubling. The yellow eye was *large*, but in double flowers the exact nature of the eye is often ambiguous. The colour was pale *mauve*. The fringe made the flowers look like *sinensis* in shape<sup>1</sup>, and the colour was taken to be of the magenta class. Subsequent breeding showed that the shape was *stellata*, and that the colour was genetically red: for crossed with red *stellata* single,  $F_1$  was red *stellata* single, and all the later history was consistent with this determination. The eye proved to be altogether peculiar. When combined genetically with singleness (Fig. 35 D) it was found to be a broad yellow eye, slightly less extensive than that of Primrose Queen (P. Q.), but the greatest extension is not central (as in P. Q.) but lateral, so that the yellow patch is in each petal bilobed. Moreover, whereas in P. Q. the style does not, in long-styled plants, rise above the anther-ring, the style of Lee's comes to the top of the tube as usual.

Both in crimping, colour, shape of flower, and eye-structure, Lee's is distinct from any variety hitherto known, and its spontaneous occurrence constitutes a mystery. Crimping in a slight degree occurs in two other varieties whose peculiarities will subsequently come up for consideration.

It seems at first that whatever variety may have been the immediate parent of the Lee's type, several factors must have been lost in its origin. This however is not a true inference. The crimp of the leaf and the fringing of the petals have, as might be expected, proved to be genetically inseparable, and are due to the loss of one factor. Also all attempts to combine the ordinary small "eye" with the crimped leaf have failed, though either form of large eye, whether the Lee's or the P. Q. sort, is compatible with it. Lastly the light pink of the corolla always has a *mauve* tinge when the leaves are crimped, so much so that accurate sorting of reds from magentas is almost impossible in a crimped class which contains both. Hence the distinctive features of Lee's may reasonably be attributed to the loss of a single factor only.

#### FORMS OF THE LEAF.

In referring to these we use the terms *palm*, *oak*, *fern*, and *crimped*. They are controlled by three allelomorphic pairs, of which none are linked to each other. All the combinations have been now made except one which is doubtless equally possible (*fern*, *crimped*).

*Palm* is the normal palmatifid shape.

<sup>1</sup> On crimped leaves we find that *sinensis* can always be distinguished from *stellata* by the possession of the much inflated calyx with ten or more teeth, not five as in *stellata*.

*Fern* (fig. 21) is a familiar recessive to it (Gregory, 1911, Pl. XXX, fig. 7). Factorially these are represented as  $P$  and  $p$ . To determine precisely the powers of  $P$  is not quite easy. In its absence the width of the leaf is much reduced and the shape becomes pinnatifid. The whole number of chief divisions of which the leaf consists is somewhat increased. Apart from minor serrations, the apical lobe is trifold in each. This in the palm is succeeded on each side by two lobes each nearly as large as the apical, posterior to which is a large lobe almost always recognizably divided into four subordinate parts, the posterior edge being free from the petiole and forming a flounce at the base of the leaf. In the fern the apical lobe is succeeded on each side by *three* lobes about as large as the apical, behind which is a series of some five or more lobes decreasing successively in size, arranged as a set of decurrent pinnae arising directly from the petiole. The whole number of lateral lobes is thus larger in the fern, being about eight on each side, as against six in the palm, counting the four subordinate divisions of the large posterior lobe separately. Of course, as in all similar examples, these numbers may differ in different plants and in the leaves of different ordinal positions, but the numerical distinction is clear in any pair of well-grown leaves occupying comparable positions. The minor serrations are also distinctly more numerous in the fern leaf. The palm and fern leaves may perhaps be said to differ somewhat as the 5-rayed limb of the mammal differs from the many-rayed fin of the fish.

*Oak* is a form known in horticulture under that name (Figs. 22 and 25). It also is a simple recessive to palm. The number of lobes is smaller than in palm. Posterior to the anterior portion, which might be taken to represent the trifold apex of the normal, there is a deep sinus which comes in nearly to the midrib. Next follows on each side a large serrated and partially bifid lobe, separated posteriorly by a still deeper sinus from a similar large and bifid lobe. This posterior lobe is always so completely separated from the rest of the leaf as to constitute an independent leaflet. The serrations are much fewer than those of the normal palm.

At the apex of each serration is a hydathode, or water-pore, which is figured in Jost's *Plant Physiology*, trans. by Gibson, Oxford, 1907, p. 57. The number of serrations is therefore that of the hydathodes. In view of what follows, information as to the exact mode of origin of these structures is desirable.

A comparison between the lobing and the serrations in palm, fern, and oak (all *flat* leaves, not crimped) is given in the following tabulation. The numbers are those of the serrations counted in a well-formed and

characteristic leaf. Each lobe is given separately (cf. Figs. 24 and 25) and the brackets indicate the grouping of the subordinate lobes into the larger ones.

Normal Palm (e.g. "Etna")			Fern			Oak		
15			13			9		
13	—	12	9	—	10	13 { 9 — 10 }	15	
17	—	14	12	—	10	4 — 5 }		
24 { 9 — 9 }			13	—	13	17 { 11 — 8 }	14	
6 — 6 }	26		17	—	13	6 — 6 }		
4 — 5 }			12	—	13			
5 — 6 }			9	—	14			
			7	—	7			
			4	—	8			
54	15	52	83	13	88	30	9	29
Total 121			Total 184			Total 68		

Fern and oak are simple recessives to palm. Fern  $\times$  oak gives  $F_1$  palm, and in  $F_2$  the combination fern-oak appears. Homozygous palm has not yet been raised from this cross, though presumably this could be done. *Fern-oak*, in addition to the apical lobe, has on each side about four large lobes and two small ones at the base. Serrations in it have been observed as follows:

	6	
6	—	7
8	—	8
9	—	9
9	—	6
2	—	3
1	—	2
35	6	35
Total 76		

Between each lobe there is a deep sinus, as in the palm-oak.

The details given by no means completely represent the leaf-shapes even of flat forms. For instance one of the palm-leaved varieties, "Ozar" (blue, with coloured gynoecium), may have as many as 165 points, but the width of the leaf in this variety is considerably greater than that of the normal and the margin lies flat. By small distinctions between the leaves, an accustomed eye could probably recognize most of the pure-breeding strains. Such details we have not attempted to analyse.

*Crimped forms.* In Lee's, which was our original crimped, the edges of the (palm) leaves bear a great number of points each with a hydathode, which commonly, if not perhaps always, is connected with a vessel. Each of these has evidently the power of independent growth uncoordinated with that of the limb of the leaf as a whole. The margin is thus enormously increased and crimping results. These little lobes have varied shapes (Figs. 26 and 27), most often forming lancet-like processes with

parallel edges, but often also widening out at the sides so that the hydathode stands as the cusp of a widely curving bracket. The processes overlap each other irregularly and the consequent crumpling of the edges of the leaf turns them downwards in greater or less degree. The normal division of the main lobes is preserved, and these are as usual one apical, and on each side two more, with a broad more or less quadripartite lobe posterior to them.

This crimping is quite distinct from that of such plants as parsley, curly kale or some forms of *Scolopendrium*. In them, owing to the multiplication of points, the edge of the leaf is much extended with consequent crumpling, but there is no development of small, minor lobes or processes as in these *Primulas*. Analogous developments may however be seen in *Begonia manicata*, var. *crispata*. *Teucrium Scorodonia* has also a somewhat similar variety, which was the subject of a paper by M. C. Rayner<sup>1</sup>.

In the original Lee's, a *stellata*, the number of points is about 630, that of a normal flat palm being about 120-130; but when the *sinensis* flower is combined with the crimped leaf the number is enormously increased. The points are then difficult to count accurately. We have counted at least 1440 in a leaf of this kind.

The combination of crimped leaves with *sinensis* flowers involves another peculiarity. When such plants are about a year old some at least of their leaves become excessively crimped, the edge of the leaf being prodigiously elongated, so that it is greatly contorted. Their leaves become so much folded as to hang almost like balls from their petioles (Fig. 36). From the fact that the points cannot be counted on the same leaf both when young and again when old, the question is difficult to decide quite positively, but we have little doubt that leaves of this combination have the power of developing new points as they grow older. On such a leaf (Fig. 19) at about 18 months old, in which the development was by no means extreme, 7000 points were counted, and doubtless much higher numbers occur. A bit of the edge of this leaf is shown in Fig. 27 magnified. The hydathodes which, when they develop, constitute the "points" are visible in such leaves at all stages of development, from slight bulges in the outline to finger-like elongations. They are *not* present in the sinuses between the chief points. Such developments have, we suppose, been studied by botanists, but we know no literature bearing on the subject.

Whether all the leaves on these plants are capable of increase in the number of hydathodes we cannot say. The change is much more striking

<sup>1</sup> This *Journal*, Vol. VII. Pl. X.

in some than in others. The appearance might perhaps be due to the development and increase in size of points already predetermined as rudimentary hydathodes, but from the presence of many still rudimentary in these old leaves, we incline to believe that an increase in number actually occurs. Nothing suggestive of this phenomenon has been seen in the leaves of any other combination.

Crimping combined with *oak* shape also increases the number of points, but in much less degree. Crimped-oak (*sinensis*) has about 96-100, instead of the 70 of flat-oak.

Besides the much crimped Lee's variety we have lately begun to work with a strain grown by Messrs Sutton which exhibits crimping in so low a degree as at first sight to be scarcely comparable. This (Fig. 30), which we call *Sutton's crimp* has about 235 points to the leaf with distinctly noticeable marginal imbrication. From the fact that this form has the same influence on the eye (though in a lower degree) as Lee's has, there is no doubt that it is essentially similar.

Under the name "Moss-curved" Messrs Sutton cultivate still a third type, with leaves crimped slightly, but in a definite and peculiar way. From various indications we infer that this variety is cytologically distinct, having an excess of chromosomes. In crosses with the normal, signs of genetical incompatibility appear. As to its factorial composition no statement can yet be made.

Though the *sinensis* flower makes an enormous difference when transferred to the Lee's crimped, giving it about 1440 instead of the 630 characteristic of the corresponding *stellata*, there is no corresponding distinction between the flat leaves when combined with either of the two flower-shapes, *sinensis* and *stellata*.

Apart from the novel phenomena of interaction to which allusion has been made, the genetical interrelationships of the leaf-shapes are not altogether what might be expected. The small number of lobes and points in palm dominates over the larger number in fern, whereas the still smaller number of divisions of both kinds in the oak is recessive to the larger number in palm. Possibly the critical distinction in the oak is really the formation of the deep sinuses, with a decrease in number of divisions consequent upon it. The curious form described by Gregory (1911, p. 87) as "ivy," which has long ago been lost, though possessing the usual number of major lobes had a smooth, undivided edge. It also was a recessive to palmate. That there should be simple dominance in all these cases is in itself remarkable. Commonly in other plants the heterozygote between a less cut type and a much cut variety is inter-

mediate. (Shull's *Capsella*; *Hibiscus* bred here, etc.) Evidently the interrelations of the *Primula* leaf-shapes are of a different nature, and the subject might repay investigation in greater detail<sup>1</sup>.

#### THE EYE OF THE FLOWER AND ITS RELATION TO CRIMPING OF THE LEAVES.

Gregory showed that the normal small yellow eye is dominant to the large eye of Primrose Queen (P.Q.). This peculiar form in which the yellow extends up over about a third of the limb of the petal was figured by him in Pl. XXX, fig. 12 (see also our Fig. 35 C). The white eye of "Queen Alexandra" (*ibid.* Pl. XXX, fig. 11, etc.), in which the yellow colour is almost totally suppressed, proved to be in varying degrees a dominant over both the other two types. Heterozygotes in this case are sometimes easily recognized as such, but by no means always. The character is interesting as the only one, besides the dominant white, which we must regard as an addition to the make-up of the species since it arrived in Europe.

The Lee's variety introduced a new type of eye, in which the yellow is only a little less extensive than in Primrose Queen, though the two can be immediately distinguished by the position of the stigma, which is normal in Lee's but below the anthers in P.Q. Lee's eye is recessive to the white eye, but is dominant over the P.Q. eye.

The white eye, the normal, and the P.Q. eye form a series of multiple allelomorphs, in this descending order (Fig. 35). They are three quantitative stages in the amount of yellow. The third term never appears in the descent from crosses of the other two, if pure. Complication is introduced by the special relations in which these forms of eye stand in regard to the crimping of the leaves. The limiting and obvious facts are:

1. The normal eye is never combined with the fully crimped leaf.
2. The P.Q. eye is compatible with the flat leaf, but the Lee's eye is not. Since the P.Q. eye has a greater extension than the Lee's this is unexpected.
3. P.Q. flat  $\times$  Lee's crimped gives  $F_1$  normal eye, flat leaf.

<sup>1</sup> Another form of the leaf exists in one of Messrs Sutton's strains of the "Duchess" colour to which they now give the name "ivy." As it is quite distinct from the old "ivy" with which Gregory worked we prefer to call it "tongue," a name which fairly well describes the shape of its elongated little-cut leaves. Petals on tongue-leaved plants are never normal, being elongated, and narrowing towards the periphery, somewhat as the leaves do. A similar alteration in the petal-shape in connexion with the leaf-shape is mentioned above as seen in the crimped forms. We have not yet worked with the tongue strain.



$F_2$  consists (7 families) of *flat* leaves, with eyes normal or P. Q.; and *crimped* leaves, with eyes Lee's or P. Q.

The cross of Lee's crimped by P. Q. flat has given  $F_2$  families as follows :

Reference Number	Flat		Crimped	
	Normal eye	P. Q. eye	Lee's eye	P. Q. eye
156/20	96	35	9	5
99/20	8	2	1	—
104/20	30	8	7	4
110/20	27	15	3	3
83/22	19	11	15	3
84/22	42	13	10	6
87/22	106	38	32	12
Totals	328	122	77	33

These numbers are very irregular, though suggesting the ratio 9:3:3:1, which gives the expectation 315:105:105:35. The subsequent history is consistent *qualitatively* with this representation inasmuch as

(1) Lee's crimped (het. in P. Q.)  $\times$  P. Q. crimped gave 74 Lee's, 54 P. Q., all crimped.

(2)  $F_1$  flat normal (from Lee's crimped  $\times$  P. Q. flat)  $\times$  Lee's crimped gave 345 flat, normal eye, 215 crimped, Lee's eye.

(3)  $F_2$  flat, normal eye (het. in P. Q.)  $\times$  flat P. Q. gave 260 normal eye, 250 P. Q. eye, all flat.

(4) Lee's eye, crimped (het. in P. Q.)  $\times$  flat P. Q. gave 62 normal eye, 72 P. Q. eye, all flat.

In each case equality of numbers is expected, from which several results depart widely. Such numerical irregularities are frequent throughout this work (see the section on this subject) but the classes produced are those we expect. In addition to those given, we have the results from various derivatives selfed, which are all confirmatory. Homozygous *flat* leaves with normal eyes have not yet been bred as derivatives from these matings, but presumably that could be done without difficulty.

From these facts we conclude that the Lee's eye is genetically identical with the normal eye, but that it is modified and extended when in combination with the crimped leaf. If this were all we might say simply that the effect of the crimped leaf is to extend the eye. Therefore we should anticipate that the P. Q. eye also, when on the crimped leaf, would be even further extended. P. Q. flowers in this combination however are *always somewhat deformed* (Figs. 11, 31, 35 E), having the edges of the petals more or less rolled inwards, with a very

narrow free limb. The appearance suggests that the eye is enlarged, but in such flowers no very strict standard of comparison can be applied.

We now meet a further complication. Since the P. Q. eye produces no noticeable effect on the normal flat leaf it was with surprise that we found that in  $F_2$  from Sutton's crimped  $\times$  P. Q. flat, those plants which combined the P. Q. eye with the crimped leaf were so much crimped as to be indistinguishable from the Lee's crimped type itself (see Figs. 31 and 28). Were it not that their eye was P. Q. and not Lee's, we might have imagined that Lee's was again newly arisen in this recombination. It should be observed that the P. Q. eye has no noticeable effect on the crimping of Lee's, but only on that of the less crimped type. At present we know no parallel to this mutual influence of two such dissimilar features upon each other.

#### THE "OLD DOUBLE" TYPE.

The late Mr E. J. Allard devoted great labour to obtaining a cross with this type. The petals are indefinite in number, succeeding each other in imbricate fashion and entirely replacing the stamens of which no trace remains. Almost always the stigma, if present, is split open, the ovules in the gynoecium being exposed. Such flowers of course produce no seed. By repeated fertilisation with singles two seeds were successfully produced on a flower in which the deformity was presumably not so extensive, and subsequently a third from a distinct cross. According to the record the male parent in the first case was a short-styled *tetraploid*<sup>1</sup>, the second having been an ordinary diploid. There is no reason to doubt the record, but if authentic it is the solitary instance of a successful cross between diploid and tetraploid plants. The strain derived from the cross was lost long since and no further evidence about the cytology exists.

This imbricate type of doubling behaved as a recessive, recurring in somewhat irregular proportions.

The original  $F_2$  consisted of 26 singles, no doubles. The absence of doubles is consistent with the belief that the male (single) was tetraploid, for only one double in sixteen would be expected. But the  $F_2$  from the cross with the single, which was certainly diploid, gave 33 singles, 2 doubles, again a notable shortage. The subsequent families in each set showed no special departure from a 3 : 1 expectation.

<sup>1</sup> Namely, a Giant. At that date no cytology had been attempted, but the strain to which this plant belonged has since been proved to be tetraploid.

The imbricate double is *possibly* linked with the style-form, whether long or short. In these double flowers, which have no anthers, the condition of the style cannot be readily told by direct observation; but since the first cross was made with a short-styled father, and longs reappeared, the double must have been genetically long-styled.

#### THE HARLEQUIN TYPE.

In  $F_2$  from the cross imbricate double  $\times$  diploid single a very peculiar new type appeared which we call *Harlequin*. In it, one, two, or rarely three of the petals are fully or almost fully coloured (whether red, magenta, or otherwise), the other petals being white or whitish. The coloured petals are of the full size, whereas the pale petals are smaller in various degrees, being sometimes much reduced in size. This type has occurred in both the *stellata* shape (Fig. 15) and also in the *sinensis* shape (Fig. 14). The deeply coloured petals, in the folding of the flower-bud, are internal. This fact suggested that possibly the pale colour of the outer petals was due to some special sensitiveness to the action of light, but flower-buds covered with black paper at an early stage showed no diminution of the "Harlequin" effect. Harlequins always breed true in their peculiarity which behaves as a simple recessive.

#### BLUE FLOWERS.

The genetical relations of blue flowers to the rest have hitherto been obscure. From the fact that plants, ostensibly blue, had been bred both from magentas and from reds, blue was regarded as recessive to those colours. It has now been established that of the flowers which we have accepted as blue, some, to which in future we shall restrict the term blue, possess a positive factor, *B*, which is absent from the others. These last we shall call *slaty*. Their colour, at least when in combination with the ordinary, light reddish stem, is recognizably distinct from that of the real blues, though on casual inspection the two may easily be confused. Microscopically examined they are seen to be perfectly distinct. In the blues the anthocyanin of the epidermis of the petals is in solution (Fig. 3), whereas most of the cells of the epidermis of slaty flowers contain anthocyanin in a solid form<sup>1</sup> (Fig. 4). Occasionally the appearance of these anthocyanin bodies suggests a crystalline structure, but more often they are amorphous. Not rarely two or more may occur together in the same cell. Sometimes dissolved anthocyanin is associated

<sup>1</sup> In the vegetative parts of these plants no solid anthocyanin has been met with.

with the solid bodies, but many cells in which they are present seem to be otherwise colourless.

It is the slaty plants which are recessive to both magenta and to red. Real blues cannot be bred from reds. Factorially the relations of the colours may be represented thus:

Magenta *BR*.

Red *bR*.

Blue *Br*.

Slaty *br*.

All the available genetical evidence is consistent with this account. The interrelations of these colours to the *coral* ("Orange King" of Gregory's paper, Pl. XXX, fig. 8) have not been completely determined, but a form nearly resembling coral exists which is recessive to slaty.

In association with the dark red stem, both the blue and the slaty are modified and assume a very peculiar appearance. This is represented in Figs. 7 and 8. No uniform blue or slaty has been seen on the dark stem, and such flowers are presumably an impossibility in that combination. The petals of such plants, both blue and slaty, apart from a uniform zone round the eye, are mottled, and the general tint is distinctly redder than that of the corresponding flowers on the light stems.

We have now to regard the factors *B* and *R* as both possessing the property of keeping the anthocyanin in solution and making its colour *blue* or *red* respectively. It should be mentioned that on fading, certain red flowers (e.g. that shown in Fig. 13) produce solid *blue* anthocyanin in the dying cells<sup>1</sup>. A peculiar type in which the petals are irrorated and mottled with red has occasionally appeared as a rare derivative from certain crosses. The general colour of these is that called strawberry (Gregory, Pl. XXXI, fig. 49 and p. 113). In one such plant which remained in the collection when these examinations were made, solid *red* anthocyanin, in part crystalline, was seen.

As a corollary to what has now been ascertained, it follows that when *magenta* has been spoken of as linked with other allelomorphs, it is really the factor *B* which should be so represented.

<sup>1</sup> Whether all have this property is not certain.

We have looked for solid anthocyanin in "blue" primroses of various tints, but found none. Mr R. J. Chittenden however called our attention to abundance of solid anthocyanin in the living epidermis of petals in a dark brown *Polyanthus*.

Gertz, quoted by Wheldale, *Anthocyanin Pigments of Plants*, 1916, p. 33, has seen solid anthocyanin in stems of *Primula sinensis*. Studier öfver Anthocyan, *Akad. Afhandling*, Lund, 1906, p. XLII.

## LINKAGE GROUPS.

Two linkage groups have been identified. The first was recognized about 1907 as a special association of *magenta* flowers with the *green* stigma. As now explained the factor which actually takes part in this linkage is *B*, blue. Subsequently it was found that short style, *S*, is included in the same linkage. To these was afterwards added *L*, the factor for the light reddish stem and leaf-backs, as opposed to its absence, *l*. Plants without *L* have these parts a deep, claret-red (see Figs. 1 and 2). Leaves of such plants, even seen from above, are intensely dark.

The four factors involved in this linkage are:

*S*, short style as opposed to *s*, long style.

*B*, blue as opposed to *b*, no blue.

(The factor *R*, causing red flowers, is probably not included in this linkage group.)

*G*, green as opposed to red stigma.

*L*, light reddish as opposed to deep red stems.

At the time of Gregory's publication coupling and repulsion had been observed as affecting this group of factors and it has since been established that they are interrelated as an ordinary linkage group. The system however exhibits one remarkable peculiarity, the first indication of which was detected by Gregory (1911, p. 128). As we now know, in two of the linkages involved, *the closeness of linkage differs greatly in the male and female sides of the same plants*. This was suspected from the  $F_2$  numbers, and has been proved by extensive back-crossing. The linkages are as follows. The values, whether as coupling or as repulsion, are the same.

	Observed linkage		Percentage of cross-overs	
	Female	Male	Female	Male
<i>SB</i>	12.2 : 1	7 : 1	7.5 %	12.5 %
<i>SG</i>	2 : 1	1.5 : 1	33.3	40
<i>SL</i>	1.7 : 1	1.47 : 1	37	40.7
<i>BG</i>	2.2 : 1	1.9 : 1	31.25	34.5
<i>BL</i>	1.8 : 1	1.7 : 1	35.6	37
<i>GL</i>	29.6 : 1	52.4 : 1	3.2	1.8

It will be noticed that the linkage of *S* with *B* is closer on the *female* side, whereas that between *G* and *L* is closer on the *male* side. We are not aware that similar sexual distinctions have been met with elsewhere. In animals the evidence is that crossing-over does not occur at all in the hetero-gametic sex, so no comparison can be instituted. In plants we are now familiar with wide differences between the genetic composition of the gametes on the two sides of heterozygous plants, but a

sexual difference in closeness of linkage has not, so far as we know, been observed.

Bridges (*Amer. Nat.* 1914, XLVIII. p. 532), taking Gregory's numbers for the linkages between the three factors here called *S*, *B*, *G*, tabulated them according to the chromosome theory. At that date reciprocal back-crosses had scarcely been undertaken and the problem to which they introduce us was not then apparent.

On the theory that crossing-over takes place during a side-to-side conjugation between the parental chromosomes this new fact presents a grave difficulty, since the loci at which the same factor must, on the theory, be supposed to stand, will be different in the male and female chromosomes. These loci will therefore not be at the same levels in the conjugating chromosomes. The hypothesis might perhaps be amended by the introduction of some conception of orderly looping in synapsis but this supposition would be difficult to verify and recourse to it would throw a considerable strain on the theory.

Judged from their microscopical appearance, the pollen grains of these *Primulas* are normal, and defective grains are as exceptional as in any pure species.

#### SECOND LINKAGE GROUP.

Linkage has also been found between the following two pairs of factors, which are not linked to any of the first group:

*F*, flat leaf as opposed to *f*, crimped leaf.

*Ch*, *sinensis* flower as opposed to *ch*, *stellata* flower.

The value of this second linkage is 8.6:1, or 10.4 % of cross-overs. This value is the same on both the male and female sides. The numbers from self-fertilisation, though showing irregularities, are not inconsistent with this estimation, though a shortage of crimped plants is sometimes conspicuous.

As regards the lower degrees of crimping, the evidence shows that "Sutton's crimped" is subject to the same linkage as the more fully crimped Lee's form.

The observed numbers, upon which these statements as to the linkages are based, will be found tabulated at the end of this paper.

#### NEW COMBINATIONS.

In the absence of the factor *G*, the gynoeceium, and especially the stigmatic surface, is coloured, being usually red, and several flower colours only reach their full development in plants which are without *G*. No fully red

petals (Fig. 12) for example, and no dark blue ones (Fig. 5) are ever formed on plants which possess  $G$ . The combination of these fully coloured flowers with the deep claret-coloured leaves, which are formed in the absence of  $L$ , had not previous to these experiments ever existed. From the horticultural point of view that combination offered great possibilities. To obtain it was one of the objects Gregory had in view, and from the theory of linkage it could readily be inferred on inspection of the  $F_2$  from  $Gl \times gl$  that the combination could be made. As the linkage is high, about 30:1 on the female side and about 50:1 on the male side, the required plant could only occur about once in 6500  $F_2$  plants. For some years no progress was made, but in 1915 a plant was noticed which had red stigmas combined with leaves somewhat redder than the ordinary "reddish" form. This was suspected of being heterozygous in  $L$ , and on selfing it produced the plants required. Usually the heterozygotes cannot be distinguished. Plants of the new combination were handed over to Messrs Sutton, and after some purification in other respects, were exhibited by them at the Royal Horticultural Society's Shows in 1921, and listed under the name "Etna." The new combination makes various others now attainable. Since this is perhaps the first practical result which has accrued from an application of the theory of linkage, the occurrence seems to be worth recording.

Unfortunately the combination of fully blue flowers with Etna foliage appears to be a physiological impossibility. Such flowers, with coloured stigmas, are characteristic of the well known variety Czar, which of course, containing  $L$ , has leaves of the lighter colour.  $Czar \times Etna$   
 $Bg Lr \quad bg lR$  gives  $F_1$  Magenta, with light foliage.  $F_2$  from this has given us the expected series Magenta, Red, Blue and Slaty on each of the two types of foliage, but as explained in the section on blue flowers, the genetically blue-flowered plants with Etna foliage have the blue colour much mottled and are scarcely recognizable as blues. The factor  $B$  in this series showed the same linkage with  $L$  as in other experiments but having been obtained after the section on linkage was prepared these families have not been brought to account.

#### A PECULIAR MOSAIC.

Colour-mosaic flowers occur sparingly. Amongst others we have for example seen magenta flowers with a radial stripe of red, or of blue, and pale magenta with a stripe of deep magenta. Each of these shows loss of a factor in the area affected, respectively  $B$ ,  $R$ , or the element for

lighter colour. Special importance attaches to a plant in two flowers of which a stripe has appeared showing the loss of *two* factors. The plant was magenta, green stigma, P. Q. eye, and the stripe in each case was the type due to the absence of *B* and *G*, being deep red of the kind seen only in combination with red stigma (see Figs. 16, 17, and 18). No flake of colour was visible in the stigma, but the gynoecium was not examined since it was hoped to raise seed from the flower.

As *B* and *G* are linked, it is of interest to observe that in this remarkable mosaic these two factors have fallen out together. The plant may be and probably is heterozygous in these two respects.

#### NUMERICAL RATIOS.

Departures from numerical expectation are common in many sections of this work. They were noticed long ago among families raised by self-fertilisation, but since, by the work of a staff, back-crossing has been rendered feasible on a considerable scale, the results so attained have emphasised the impression previously formed. Adequate treatment of this subject is beyond the scope of this paper. It would involve much tabular printing and the application of statistical methods with which we are unfamiliar.

A few specimens of the numbers we have met with are included, with a report on them most kindly supplied by Dr G. Udny Yule, F.R.S.

To have much value such an investigation should deal collectively with the numbers recorded for divers characteristics in a great variety of organisms. Experience of such numbers suggests that, apart from numerical aberrations due to differential mortality and comparable interferences, there are significant distinctions between various organisms in this respect, some following closely, others departing more often from the numerical equality which may be regarded as the normal consequence of simple Mendelian segregation. We are disposed to attribute some at least of these departures to definite sporadic events whereby, of the two kinds of gametes with contrary powers, one has become more numerous than the other. It can happen but rarely in practice that more than one breeding test can be applied to the same aberrant plant. We have only one instance which supplies such evidence. Three families were raised by self-fertilisation from apparently similar plants, heterozygous in stigma colour as follows:

1922		Green stigma	Red stigma
Number 39	gave	109	33
• Number 42	„	125	45
Number 44	„	100	8



39 and 42 may each be taken as obviously 3:1, but 44 gave about 12:1. It happens that the parents which produced these offspring by self-fertilisation were also back-crossed with the recessive, giving the following results:

		Green stigma	Red stigma
39 <sup>3</sup> /21	parent of 39/22 as ♀	...	27
"	" " as ♂	...	29
39 <sup>4</sup> /21	parent of 42/22 as ♂	...	9
	(not tried as ♀)		14
40 <sup>1</sup> /21	parent of 44/22 as ♀	...	62
"	" " as ♂	...	39
			15

These figures prove that the parent of 39/22 was normal on both sides in the equality of gametes bearing the two allelomorphs, that the parent of 42/22 may have been similarly normal, but that the parent plant from which 44/22 was derived was almost certainly abnormal on both sides.

Taking 62:22 as probably indicating 3:1 on the ♀ side and 39:15 as suggesting 2:1 on the ♂ side, we should expect a ratio of 11:1 on self-fertilisation, which is approximately the ratio produced (99:9 where 100:8 was observed).

Usually such aberrations are noticed too late for any check to be applied. In cases of abnormal ratios we have often tested the resulting plants, but have found no recurrence of the aberrant numbers. Gregory (1911, pp. 84-5) records however an instance of this kind where the short-style gametes were greatly in excess throughout a related group of plants<sup>1</sup>.

As specimens of the general run of the numbers we give three examples. Dr Udney Yule has examined these series and prepared a report on each which we are permitted to incorporate. The first relates to plants heterozygous in three pairs of factors, *sinensis*—*stellata*; green stigma—red stigma; white ("Alexandra") eye—ordinary yellow eye. All were back-crossed with triple recessives. In 16 families the heterozygote was the mother, in 6 the father.

The results are given in tabular form. Simple expectation of course is that the eight numbers in each column should be equal. The families 110 and 131 are especially abnormal. Gregory devoted much study to such examples. In his opinion they strongly suggested that whether by successive segregation followed by proliferation in special groups of

<sup>1</sup> On p. 125, *ibid.* we find there was an error in copying from the record. In the last line the figure "17" should have been "10," which weakens the argument advanced in the text.

*Triple heterozygote ♀ × triple recessive ♂.*

Reference Number	54	55	58	59	107	110	119	121	122	127	129	131	132	133	135	178	Totals
Sin.	{	gr. stig.	{	wh. eye	5	18	17	2	12	17	9	10	24	9	11	10	192
		{	yell. eye	4	10	17	3	20	16	10	7	23	3	6	24	18	188
		red stig.	{	wh. eye	13	22	20	10	14	10	6	8	19	5	5	23	202
Stall.	{	gr. stig.	{	yell. eye	10	11	12	6	13	13	9	8	9	6	3	12	152
		{	wh. eye	10	13	11	12	5	5	16	2	30	3	8	21	19	180
		red stig.	{	yell. eye	9	17	11	11	12	6	14	3	16	5	7	13	165
	{	wh. eye	14	16	18	9	7	3	18	2	11	5	4	14	23	4	167
		{	yell. eye	7	12	16	6	10	8	10	4	23	5	4	22	23	181
Totals	...	72	119	122	59	93	78	92	44	155	41	40	145	153	41	76	1427
Value of <i>P</i>		.20	.35	.60	.072	.085	.0076	.18	.090	.012	.68	.24	.87	.048	.65	.96	

*Triple heterozygote as ♂ crossed with triple recessive ♀.*

Reference Number	60	63	64	94	95	97	Totals				
Sin.	{	gr. stig.	{	wh. eye	11	11	10	28	7	7	74
		{	yell. eye	16	4	5	28	4	4	7	64
	{	red stig.	{	wh. eye	18	13	15	24	2	10	82
		{	yell. eye	8	12	11	26	5	5	7	69
Stell.	{	gr. stig.	{	wh. eye	10	8	6	19	6	14	63
		{	yell. eye	9	7	11	29	10	12	78	
	{	wh. eye	10	12	12	26	4	11	75	79	
		{	yell. eye	13	14	14	24	7	7	79	
Totals	...	95	81	84	204	45	75	584			
Value of <i>P</i> ...		.393	.317	.317	.898	.387	.570	.695			

segregates, or by some other process, significant inequalities in the resulting numbers were produced not uncommonly. Whatever the source of the inequalities, we are disposed to regard them as representing a definite physiological phenomenon. In 110 for example the *sinensis* group (56) is more than double the *stellata* group (22), and we think it likely that either proliferation has occurred in the one or an inhibition of division in the other, such that the ovary was probably in a state analogous to that of a mosaic plant or branch.

Nevertheless we see no indication of regularity among these aberrant numbers. Sometimes one group, sometimes another is in excess; nor, on the analogy of mosaicism, would regularity be expected. Dr Yule reports as follows:

"The chances for the several families of getting a series of deviations from expectation (uniformity) as bad as or worse than those observed (obtained by the  $\chi^2$  method) are as follows:

Female heterozygote		Male heterozygote	
54	·20	60	·40
55	·35	63	·32
58	·60	64	·32
59	·072	94	·90
107	·085	95	·39
110	·0076	97	·57
119	·18		
121	·090		
122	·012		
127	·68		
129	·68		
131	·24		
132	·87		
133	·048		
135	·65		
178	·96		

The first series of families, in which the female was the heterozygote, is clearly significantly divergent from expectation. A family as badly divergent as 110 would only be expected on random sampling once in some 1300 trials. If sampling were random the values of the above chance, usually denoted by  $P$ , should be uniformly distributed over the range 0 to 1. They are not at all uniformly distributed and 6 of the values are less than 0·1, whereas only 1 or 2 (one-tenth of 16 or 1·6) should be less than 0·1. The average value of  $P$  for this series is only 0·358 instead of 0·5.

The second series, in which the male was heterozygous, shows no evidence of anything abnormal: no value of  $P$  is very low, and the average is 0·48, or very near the theoretical 0·5.

For the totals of the first group of families  $P$  is 0.17, for the totals of the second group 0.69. This is confirmatory of the conclusion as to the greater abnormality of the first group. It looks as if the abnormalities were in the ovules rather than the pollen.

Reverting to the first series of families, it will be seen that while family 131 catches the eye owing to the regular distribution of its divergences, the value of  $P$  is not very low (0.24). But if the distribution of the characters for each pair ( $A$ , sin:  $B$ , stigma:  $C$ , eye) are taken out, these distributions, and the ratios for the single characters, are all very near the expected equality. The oddity only comes out when the three characters are taken together.

When family 110 is taken in the same way it will be seen that the ratio of  $A : a$  is altogether abnormal (56 : 22); you would not expect such a divergence on random sampling more often than once in some 8000 trials or so, but the ratios for  $B$  and  $C$  are much nearer normality. Correspondingly, for the three pairs of characters in this family the values of  $P$  are

$AB$  .00061

$AC$  .0012

$BC$  .60

$A$  (the sin: stell pair) is apparently the source of abnormality.

Similar investigation of family 122 suggests that  $B$  (stigma) is the source of abnormality. The ratio  $B : b$  is 93 : 62 and the values of  $P$  are low for the pairs  $AB$  and  $BC$  (.085 and .028) but quite high for  $AC$  (.63).

Family 133 is small and one would hardly expect to get anything very clear out of it, but  $C$  (eye) looks like the source of trouble. The ratio  $C : c$  is 27 : 14 and the pairs  $AC$ ,  $BC$  give the lowest values of  $P$ , the pair  $AB$  being quite a good fit. It is a curious accident that, taking these three abnormal families, each suggests an abnormality arising from one of the three characters only, and a different character in each case. There are probably not more than 4 or 5 abnormal families out of the 16 altogether, judging from the run of the  $P$ 's—assuming of course that the families can be definitely sundered into normal and abnormal."

We also submitted to Dr Yule the records (too extensive for publication here) of 211 families in which plants heterozygous for short-style (thrum)—long-style (pin) had been back-crossed with recessives, and of 331 families similarly tested for magenta—red. Many of the heterozygous parents are common to both series, and they represent tests of both ovules and pollen indiscriminately. He has furnished the following report:

"The *totals* cannot be regarded as diverging significantly from equality though the divergences are a little uncomfortably large: the number of thrums differs from expectation (4090) by 1.79 times the standard error and the number of magentas differs from expectation (6739.5) by 1.65 times the standard error. But in view of the fact, which comes out very clearly on examining the figures for corresponding families in the two records, that *S* and *B* are highly linked if the one character shows a rather large divergence from expectation the other is almost bound to: the results are not independent.

I have examined the data as regards the fluctuation of the proportion of dominants amongst the families. I sorted out the families with 50 plants or more [52 for thrum—pin; 99 for magenta—red], listed them separately, worked out the percentage of dominants in each, and booked up the frequency distributions, which are given in the table below. The

Number of families showing said percentage of			Number of families showing said percentage of		
Percentage	<i>S</i>	<i>B</i>	Percentage	<i>S</i>	<i>B</i>
37	1	—	55	3	1
38	—	1	56	6	4
39	1	—	57	—	6
40	—	2	58	—	3
41	1	5	59	1	5
42	1	2	60	1	2
43	1	4	61	—	2
44	4	6	62	—	1
45	—	1	63	—	1
46	1	3	64	—	—
47	6	9	65	—	1
48	1	4	66	1	—
49	2	5	67	—	—
50	7	6	68	—	1
51	4	7	69	1	—
52	3	10	70	—	—
53	4	4	71	—	1
54	2	2			
Totals			52	99	

standard deviations of these distributions compare as follows with the standard deviations of sampling:

	Observed S.D.	S.D. of sampling
Thrum ...	5.97	5.76
Magenta ...	6.59	5.79

In both distributions the observed standard deviation is greater than the S.D. of simple sampling, the excess being the more marked for the 'Magenta' series, i.e. the fluctuation is rather greater than is theoretically expected.

As regards the form of the frequency distributions, the only thing to be noted is that they are exceedingly irregular, as will be seen from

the table. The *similarity* of the peaks in the two distributions suggested some significance, but this is probably only due to the linkage and the fact that the 'Magenta' distribution contains the majority of the families in the 'Thrum' record. There is an odd symmetry about the peaks however. If the distribution is doubled over round 50 per cent. so as to add the frequencies for 51 and 49 per cent., 52 and 48 per cent. and so on, the peaks stand out even more clearly than before.

As regards specially exceptional families, I have not made a detailed examination, but 106/12 [64*B*, 30*b*] and 103/12 [76*B*, 114*b*] in the 'Magenta' series caught my eye. The chance of such a divergence from equality occurring is about .0005 for the first and .006 for the second. The small family 38/12, with 15*B* and 2*b* only, is also very divergent—the chance of such a divergence occurring on random sampling being only about .0023.

There is nothing, unfortunately, clear-cut and definite—but, wider fluctuation than there ought to be, distributions irregular, and irregularities slightly suggestive of something definite, and some markedly exceptional families, and that seems about all one can say."

Pending a comprehensive examination of such numbers collected from various sources it is, as we have said, not possible to assert positively to what degree those that we have given are unusual, but we are inclined to think that the amplitude of divergence from normality differs considerably in the various subjects studied and perhaps also in regard to special factors. As the comparison with mosaics may naturally be made, it should be added that mosaics in the somatic tissues are by no means common in *P. sinensis*. An occasional flake of a recessive colour in the flower is not very uncommon and sometimes *stellata* petals may appear on plants heterozygous for *sin.—stell.*, but even these mosaics in general are rare. A flower mosaic in colour is figured (Fig. 16) and discussed on p. 233.

#### THE HISTORY OF *PRIMULA SINENSIS*.

In view of the genetical interest of the species an accurate account of its origin is greatly to be desired. Unfortunately little is positively known as to the circumstances in which it was first seen in China, and we have no acceptable surmise as to its wild progenitor or progenitors. The first evidence of the existence of such a plant reached Europe in the form of a drawing received from China by the Royal Horticultural Society in 1819. At their request seeds and a plant were dispatched by Mr [John] Reeves who procured them in Canton, but the plant died

and the seeds failed to germinate. Shortly after, a plant was brought over successfully by Capt. Richard Rawes "from gardens" at Canton.

In 1821 two coloured plates were published by Ker-Gawler (*Bot. Reg.* Pl. 539) and by Lindley (*Collectanea Botanica*). Whether both represent the original plant is not clear. More probably they were made from its immediate offspring. Both show the flowers as magenta of the shade associated with a green stigma, and both in flower-shape approach *sinensis*, though to an observer accustomed to these plants, they are almost certainly of the type called by Messrs Sutton "*pyramidalis*," viz. heterozygous in respect of the *stellata* shape. Being *sinensis*, they have ten calyx-teeth, and Lindley had some doubt whether, in view of this peculiarity, the plant was rightly referred to the genus *Primula*. In accordance with the ideas of the period the suggestion was made in *Bot. Reg.* that this might be the effect of "luxuriance"!

W. J. Hooker (*Exotic Flora*, 1825, Vol. II. Pl. 105) gives a very good coloured figure, this time of the *stellata* type with five calyx-teeth, and a less satisfactory drawing of a similar plant was published in *Bot. Mag.* 1824, tab. 2564. Mention is made of the *sinensis* type with ten calyx-teeth as coexisting with the *stellata* form. Of the plants figured in these four plates some were certainly and all may have been short-styled. In all the leaves are palm<sup>1</sup>.

Another early reference is made by Lindley (*Trans. Hort. Soc.* VI. 1826, p. 80) who, after speaking of Rawes's first importation, states that plants raised from seeds afterwards brought from China by Mr Potts, one of their collectors<sup>2</sup>, were distributed by the Society.

This second importation is not usually quoted in histories of the plant. Since, however, Lindley in 1826 explicitly states that the plant was known to him in two varieties [*sinensis* and *stellata*] we may be fairly sure that nothing ostensibly fresh was raised directly from Mr Potts's seeds.

The original plant therefore had nearly all the dominant factors yet identified. Two only have been gained since: the dominant white which inhibits the formation of anthocyanin in the petals, and the white or "Alexandra" eye which inhibits the yellow of the normal eye. The white eye was introduced by MM. Vilmorin about 1902, but no details are known as to the origin of either of these two dominants.

As regards variation by loss, it is clear that the *stellata* form appeared

<sup>1</sup> The leaves in Lindley's plate, said to have been drawn by W. J. Hooker, are curiously different from any we know. Probably this is due to imperfect drawing.

<sup>2</sup> The Librarian of the R. Hort. Soc. kindly showed us a MS. diary of Mr Potts's Chinese experiences, but we found in it no mention of *Primula*.

at once, the original being heterozygous for it. The same is probably true of the long-style. Good notes of the cultural history are given by A. W. Sutton<sup>1</sup> with dates at which novelties were noticed or developed. Many were the result of deliberate cross-breeding, especially those brought out by Messrs Sutton, one being especially noteworthy—the “Duchess” type—as evidently due to a breaking up of the dominant white. It has petals white peripherally with a red band round the eye. This is figured by Gregory (1911, Pl. XXXI, figs. 27–8). As he stated, the periphery of the corolla alone is white, the complementary part of the dominant-white complex which controls the gynoeceum and the centres of the petals being absent. “Duchess” cannot therefore exist with a green stigma, for that would enable the white to invade the centre of the flower.

Usually little can be established as to the variations by which the original factorial composition has been changed. The fern-leaf appeared early in the cultivated history, and the crimped or crisped leaf several times. Perhaps the clearest evidence relates to the colour known as “orange” or “coral,” which is known to have come by loss of a single factor, without crossing, from the crimson called “Crimson King,” a type which had been bred, on a very large scale, true for many years. Blues, derived from magenta by loss of the factor *R*, and the large “Primrose Queen” eye, are among the latest recessives to appear.

The question arises, what were the plants first seen by Mr. Reeves in China, and whence did they come? The statement made in *Bot. Reg.* is that Capt. Rawes brought the plant “from gardens at Canton, where it probably found its way from some far more northern quarter of the country.” This implies that *P. sinensis* was then already in cultivation by the Chinese, but repeated inquiries from competent botanists acquainted with China have failed to elicit anything as to such plants being at present in cultivation there. Mr. W. Tutchet of the Botanical and Forestry Dept., Hong Kong, kindly wrote (1910) that old gardeners there remembered growing it thirty years before, but can add nothing more. We have also letters, which Dr A. Henry and Mr George Forrest were good enough to send us, containing negative information<sup>2</sup>. Dr Henry directed us to a figure in a modern Chinese Botany, *Chih Wu Ming*, Vol. xxix. p. 18; which might possibly represent the *stellata* form. We are obliged to Mr Waley and Mr Giles of the British Museum for a translation of the text, which is unfortunately inconclusive. The plant in

<sup>1</sup> *J. R. Hort. Soc.* 1891, Vol. xiii. p. 99.

<sup>2</sup> A letter to the same effect has lately been received from Mr F. Kingdon Ward, well known for his collections of Chinese plants.



question was from Yunnan. Its leaves and bracts are described in terms suggestive of *sinensis* rather than of any other species that we know, but till some collector finds this plant, its nature must remain ambiguous.

As the originals were in "gardens" they *may* have had a history of hybridization behind them. The production of such a series of novelties coming into existence within so few generations is scarcely to be paralleled by any pure-bred species of plant. On the other hand there is no indication of infertility at any time (except in connexion with tetraploidy), and though varieties came, they seem to have appeared sporadically over a period of years. Disintegration consequent on hybridization is a very different process.

The nearest parallel is perhaps to be seen in the Sweet Pea (*Lathyrus odoratus*), though in it the structural variation has been very much less. *Primula obconica*<sup>1</sup> also has given rise to several types differing chiefly in colour and size, but nothing approaching the multitude of forms known in *P. sinensis* has appeared, and *P. malacoides*<sup>1</sup>, though it also has produced varieties, is by comparison a fixed species.

The history of the three species of *Primula* agrees moreover in the fact that though many crosses have been tried with all of them not one is known to have been successful. The various records of alleged positive results are almost certainly erroneous, and there is scarcely a doubt that these plants are all genuine examples of spontaneous variation, almost always by loss of factors, occurring without crossing, under domestication. To those familiar with modern genetics it is scarcely necessary to point out that spontaneous variation is not the common occurrence we formerly thought it to be, and in the history of cultivated plants the Sweet Pea and *P. sinensis* stand out as probably the two best authenticated examples of this phenomenon manifested on a large scale.

We used to regard the Sweet Pea as a plant above suspicion of having undergone crossing. Trials with numerous species of *Lathyrus* are known to have resulted in failure. Successes have been reported from time to time but the accounts have been insufficient, and in view of the species used<sup>2</sup>, and the ease with which errors may occur, unconvincing. In 1916, however, Barker<sup>3</sup> gave a full account of a cross

<sup>1</sup> Evidence collected by A. W. Hill, as to *P. obconica*, *Jour. Gen.* 1913, Vol. II. p. 1, 2 Plates; and as to *P. malacoides*, *ibid.* 1918, Vol. VII. p. 193, 2 Plates.

<sup>2</sup> For example, a cross with *L. pratensis*, *Gard. Chron.* 1913, I. p. 173; with "*L. luteus aureus*," *ibid.* p. 85.

<sup>3</sup> *Gard. Chron.* 1916, p. 156. Mr S. C. Harland recently informed us that he has independently made this cross and has  $F_2$  seeds.

between Sweet Pea Kitty Clive fertilised by *L. hirsutus*, which produced fertile  $F_1$  plants, giving segregation at least in colour and size in  $F_2$ . Any chance that the Sweet Pea could have been crossed during its early history with *hirsutus* or any other species is however so remote that we need scarcely hesitate to accept its variations as the spontaneous developments of a pure species.

As regards the Sweet Pea, *P. obconica* and *P. malacoides*, there is no difficulty in tracing them to the single wild original species. Till some wild species is found from which *P. sinensis* can have been derived, we cannot be perfectly certain that no crossing has occurred, but the presumption is against that supposition.

Of the vast number of wild species which have been brought from China none really resembles *sinensis* to the eye of any one intimate with that plant. Mention must be made of a curious mistake which was made in this respect. A species was found by Mr Walters and later by Dr Henry and the Abbé Delavay at Ichang on the Yang-tze river, which was taken first by Dr Masters and afterwards by other distinguished botanists for the original *P. sinensis*. Under that name it was figured in *Bot. Mag.* (1897, tab. 7559) where many particulars are given. No one seems to have doubted about it. There are nevertheless definite differences. The shape of the leaf is quite distinct from all the leaf-shapes known in *sinensis* both in lobing and the crenulations of the margin. The leaves are hard to the touch. Their surface has a fine very short and even pubescence, not the pilose ciliation of *sinensis*. The bracts and calyx are also different, and the scent of the foliage is quite distinct<sup>1</sup>. Besides these features which can be expressed in words, there is a difference in substance and general appearance which to those accustomed to handle *sinensis* is very clear, and though *a priori* no one could deny that the Yang-tze species might conceivably have been one of the parents of *sinensis*, there is no question of identity. It is not even surprising to us that all attempts to cross that species with *sinensis* have failed. The capsules swell but no seed is formed. With its own pollen it has so far bred true to type. Cytological examination has not yet been made.

<sup>1</sup> Thé *Bot. Mag.* plate shows the petals covered with hairs. These however are not present in the plant. They appear, though less conspicuously, in the original drawing which Mr A. W. Hill has shown us and must have been put in by mistake. In the plant (as in *sinensis*) there are glandular hairs on and close to the yellow eye, but the limb of the petals is glabrous.

Since this was written the name *Primula calciphila* has been proposed for the species in question (see *Gard. Chron.* 1923, p. 101, Fig. 49).

Subsequently a species was collected by Farrer<sup>1</sup> which has a somewhat closer resemblance, such indeed that we anticipated that crosses with it might succeed. We have not had this plant, but crosses tried at Kew have failed.

Finally we must recognize the problem created by the *ten-toothed* calyx associated with the *sinensis* shape of the flower as originally introduced. Ker-Gawler compared the flower to that of *P. cortusoides*, but it seems that no wild species is yet known to have the ten calyx teeth. This depends on the presence of a definite dominant factor. The heterozygote, though often recognizable as such, is so like pure *sinensis* that batches of the pure and heterozygous forms cannot be sorted with confidence. The *stellata* by contrast is—when combined with a flat leaf—clearly distinguishable. We must therefore admit the likelihood that this dominant *sinensis* factor has been added since domestication though before the plant left China.

#### NUMBERS OBSERVED IN THE LINKAGE SERIES.

##### *First Linkage Group.*

Factors involved:

*S*, short style in the absence of which the style is long (*s*).

*B*, blue flowers in the absence of which the flower is slaty (*b*)

[unless *R* the factor for red is present].

*G*, green stigma and gynoeceium in the absence of which these parts have anthocyanin (*g*).

*L*, stems and leaf-backs light red in the absence of which these parts are deep red (*l*).

On the chromosome theory the "order of the genes" would be as above, *S*, *B*, *G*, *L*.

♀  $\frac{SB}{sb} \times sb$

*Data for linkage SB\*.*

	<i>SB</i>	<i>sB</i>	<i>sB</i>	<i>sb</i>	
	120	17	10	109	
	1250	102	82	1287	from matings involving also <i>G</i>
	152	16	24	159	" " " <i>L</i>
	146	13	12	148	" " " <i>G</i> and <i>L</i>
Totals	1668	148	128	1703	

*Linkage on female side 12:2 : 1, or 7.5% of cross-overs.*

\* The data include those previously published by Gregory. It should be understood that numbers taken from matings involving factors other than those dealt with in any particular group reappear also in the groups relating to those other factors.

Further data on the linkages between *S*, *B* and *G* were obtained by Altenburg (*Genetics*, Vol. I. p. 354 and *Amer. Nat.* 1921, LV. p. 78) on material supplied by Gregory. These figures are not here included.

<sup>1</sup> It has received the name *P. rupestris*, Balf. f. et Farrer. J. Bayley Balfour, *Trans. Bot. Soc. Edin.* 1918, Vol. XXVII. p. 240.

$\text{♀ } sb \times \frac{SB}{sb}$				
	<i>SB</i>	<i>Sb</i>	<i>sB</i>	<i>sb</i>
	147	20	20	157
	770	102	106	643
	586	72	83	580
	from matings involving also <i>G</i>			
	,, ,, ,, <i>G</i> and <i>L</i>			
Totals	1503	194	209	1380

Linkage on male side 7 : 1, or 12.5 % of cross-overs.

$\frac{SB}{sb}$ selfed				
	<i>SB</i>	<i>Sb</i>	<i>sB</i>	<i>sb</i>
	156	18	11	57
	72	2	4	15
	198	12	8	61
	1897	91	122	620
	also involving <i>G</i>			
	,, ,, <i>L</i>			
	,, ,, <i>G</i> and <i>L</i>			
Totals	2323	123	145	753
Expectation	2346	159.7	159.7	675.5
Calculated on	$\left\{ \begin{array}{l} \text{♀ linkage } 12.2 : 1. \\ \text{♂ } ,, 7 : 1. \end{array} \right.$			

*Data for linkage SG.*

$\text{♀ } \frac{SG}{sg} \times sg$				
	<i>SG</i>	<i>Sg</i>	<i>sG</i>	<i>sg</i>
	70	35	45	56
	896	430	440	914
	99	60	66	98
	also involving <i>B</i>			
	,, ,, <i>B</i> and <i>L</i>			
Totals	1065	525	551	1068

Linkage on female side 2 : 1, or 33.3 % of cross-overs.

$\text{♀ } sg \times \frac{SG}{sg}$				
	<i>SG</i>	<i>Sg</i>	<i>sG</i>	<i>sg</i>
	31	28	21	33
	535	331	315	428
	391	267	266	397
	also involving <i>B</i>			
	,, ,, <i>B</i> and <i>L</i>			
Totals	957	626	602	858

Linkage on male side 1.5 : 1, or 40 % of cross-overs.

$\frac{SG}{sg}$ selfed				
	<i>SG</i>	<i>Sg</i>	<i>sG</i>	<i>sg</i>
	49	15	10	7
	60	14	11	8
	287	70	83	55
	also involving <i>B</i>			
	,, ,, <i>B</i> and <i>L</i>			
Totals	396	99	104	70
Expectation	401.4	100.3	100.3	66.9
Calculated on	$\left\{ \begin{array}{l} \text{♀ linkage } 2 : 1. \\ \text{♂ } ,, 1.5 : 1. \end{array} \right.$			

	$\frac{Sg}{sG}$ selfed				
	<i>Sg</i>	<i>Sg</i>	<i>sG</i>	<i>sg</i>	
	1367	537	532	114	also involving <i>B</i> and <i>L</i>
Expectation	1360	552.5	552.5	85	

Repulsion calculated as above.

*Data for linkage SL.*

	$\frac{SL}{sl} \times sl$				
	<i>SL</i>	<i>Sl</i>	<i>sL</i>	<i>sl</i>	
	212	125	130	234	
	90	58	54	88	also involving <i>B</i> and <i>G</i>
Totals	302	183	184	322	

Linkage on female side 1.7 : 1, or 37% of cross-overs.

	$\frac{SL}{sl} \times \frac{SL}{sl}$				
	<i>SL</i>	<i>Sl</i>	<i>sL</i>	<i>sl</i>	
	72	56	57	102	
	387	265	273	387	also involving <i>B</i> and <i>G</i>
Totals	459	321	330	489	

Linkage on male side 1.47 : 1, or 40.7% of cross-overs.

	$\frac{SL}{sl}$ selfed				
	<i>SL</i>	<i>Sl</i>	<i>sL</i>	<i>sl</i>	
	99	35	32	12	also involving <i>B</i>
	1578	375	549	230	„ „ <i>B</i> and <i>G</i>
Totals	1677	410	581	242	
Expectation*	1727.7	454.8	454.8	272.7	

Calculated on  $\left\{ \begin{array}{l} \text{♀ linkage } 1.7 : 1. \\ \text{♂ „ } 1.47 : 1. \end{array} \right.$

	$\frac{SL}{sL}$ selfed				
	<i>SL</i>	<i>Sl</i>	<i>sL</i>	<i>sl</i>	
	273	84	126	12	also involving <i>B</i> and <i>G</i>
Expectation*	266	105.2	105.2	18.6	

Repulsion calculated as above.

\* The departures from expectation on selfing are here and elsewhere perhaps noteworthy in view of the comparative regularity of the results of back-crossing.

*Data for linkage BG.*

	$\frac{BG}{bg} \times bg$				
	<i>BG</i>	<i>Bg</i>	<i>bG</i>	<i>bg</i>	
	1186	509	511	1051	
	926	381	410	963	involving also <i>S</i>
	202	93	112	176	„ „ <i>L</i>
	140	69	66	142	„ „ <i>S</i> and <i>L</i>
Totals	2454	1052	1099	2332	

Linkage on female side 2.2 : 1, or 31.25% of cross-overs.

*Genetics of Primula sinensis*♀  $bg \times \frac{BG}{bg}$ 

	<i>BG</i>	<i>Bg</i>	<i>bG</i>	<i>bg</i>	
	247	120	141	250	
	584	267	284	474	involving also <i>S</i>
	96	50	59	90	„ „ <i>L</i>
	423	241	231	417	„ „ <i>S</i> and <i>L</i>
Totals	1350	678	715	1231	

Linkage on male side 1.9 : 1, or 34.5% of cross-overs.

 $\frac{BG}{bg}$  selfed

	<i>BG</i>	<i>Bg</i>	<i>bG</i>	<i>bg</i>	
	1691	491	421	324	
	62	14	9	8	involving also <i>S</i>
	413	92	110	80	„ „ <i>L</i>
	304	67	66	58	„ „ <i>S</i> and <i>L</i>
Totals	2470	664	606	470	
Expectation	2578.9	578.4	578.4	474.1	

Calculated on  $\left\{ \begin{array}{l} \text{♀ linkage } 2.2 : 1. \\ \text{♂ „ } 1.9 : 1. \end{array} \right.$  $\frac{Bg}{bG}$  selfed

	<i>BG</i>	<i>Bg</i>	<i>bG</i>	<i>bg</i>	
	1332	488	553	100	involving <i>S</i> and <i>L</i>
Expectation	1303.1	551.6	551.6	66.6	

Repulsion calculated as above.

*Data for linkage BL.*♀  $\frac{BL}{bl} \times bl$ 

	<i>BL</i>	<i>Bl</i>	<i>bL</i>	<i>bl</i>	
	238	124	149	275	
	105	71	64	123	involving also <i>S</i>
	197	98	119	169	„ „ <i>G</i>
	97	50	47	96	„ „ <i>S</i> and <i>G</i>
Totals	637	343	379	663	

Linkage on female side 1.8 : 1, or 35.6% of cross-overs.

♀  $bl \times \frac{BL}{bl}$ 

	<i>BL</i>	<i>Bl</i>	<i>bL</i>	<i>bl</i>	
	50	31	28	52	
	92	63	53	87	involving also <i>G</i>
	423	241	237	411	„ „ <i>S</i> and <i>G</i>
Totals	565	335	318	550	

Linkage on male side 1.7 : 1, or 37% of cross-overs.

$\frac{BL}{bl}$ selfed				
	<i>BL</i>	<i>Bl</i>	<i>bL</i>	<i>bl</i>
	94	35	37	12
	42	10	10	6
	1462	403	452	243
	involving also <i>S</i>			
	" " <i>G</i>			
	" " <i>S</i> and <i>G</i>			
Totals	1598	448	499	261
Expectation	1686.9	417.5	417.5	283.9
Calculated on $\left\{ \begin{array}{l} \text{♀ linkage } 1.8 : 1. \\ \text{♂ } \quad \quad 1.7 : 1. \end{array} \right.$				

$\frac{Bl}{bL}$ selfed				
	<i>BL</i>	<i>Bl</i>	<i>bL</i>	<i>bl</i>
	286	85	113	11
	involving also <i>S</i> and <i>G</i>			
Expectation	263.7	107.3	107.3	16.4

Repulsion calculated as above.

*Data for linkage GL.*

$\text{♀ } \frac{GL}{gl} \times gl$				
	<i>GL</i>	<i>Gl</i>	<i>gL</i>	<i>gl</i>
	276	6	12	269
	305	11	9	253
	189	4	5	142
	involving also <i>B</i>			
	" " <i>S</i> and <i>B</i>			
Totals	720	21	26	669

Linkage on female side 29.6 : 1, or 3.2% of cross-overs.

$\text{♀ } gl \times \frac{GL}{gl}$				
	<i>GL</i>	<i>Gl</i>	<i>gL</i>	<i>gl</i>
	254	8	4	208
	142	4	3	146
	647	7	13	645
	involving also <i>B</i>			
	" " <i>S</i> and <i>B</i>			
Totals	1043	19	20	999

Linkage on male side 52.4 : 1, or 1.8% of cross-overs.

$\frac{Gl}{gL}$ selfed				
	<i>GL</i>	<i>Gl</i>	<i>gL</i>	<i>gl</i>
	986	499	418	—
	1809	828	851	—
	involving also <i>B</i> , or <i>S</i> and <i>B</i>			
Totals	2795	1327	1269	—
Expectation	2696.2	1348.2	1348.2	0.8

Repulsion calculated on  $\left\{ \begin{array}{l} \text{♀ linkage } 29.6 : 1. \\ \text{♂ } \quad \quad 52.4 : 1. \end{array} \right.$

The tabulations given above include all the numbers bearing on the linkage actually observed. In the following tables the same families, in

so far as they are applicable, are arranged for the calculations of linkages between the same factors *S*, *B*, *G*, *L*, separately for the combinations of any three of them taken together, and collectively for all four. All the expectations are calculated on the linkages determined above, by the application of Trow's method.

$\frac{SBG}{sbg} \times sbg$								
	<i>SBG</i>	<i>sbg</i>	<i>SBg</i>	<i>sbG</i>	<i>Sbg</i>	<i>sBG</i>	<i>SbG</i>	<i>sBg</i>
	960	977	402	427	85	65	27	27
Expectation	943.3		428.8		77.3		35.15	
$\frac{SBG}{sbg} \times \frac{SBG}{sbg}$								
	<i>SBG</i>	<i>sbg</i>	<i>SBg</i>	<i>sbG</i>	<i>Sbg</i>	<i>sBG</i>	<i>SbG</i>	<i>sBg</i>
	870	771	475	444	119	137	54	51
Expectation	837.2		440.6		119.6		62.9	
$\frac{SBG}{sbg}$ selfed								
	<i>SBG</i>	<i>sbg</i>	<i>SBg</i>	<i>sbG</i>	<i>Sbg</i>	<i>sBG</i>	<i>SbG</i>	<i>sBg</i>
	340	49	78	68	6	21	8	3
Expectation	329.2	52.1	72.6	63.6	12.3	21.4	15	6
$\frac{SBg}{sbG}$ selfed								
	<i>SBG</i>	<i>sbg</i>	<i>SBg</i>	<i>sbG</i>	<i>Sbg</i>	<i>sBG</i>	<i>SbG</i>	<i>sBg</i>
	1249	85	460	483	15	83	70	28
Expectation	1210.1	53.8	529.9	445.5	12.4	91.2	105.4	26.9
$\frac{SBL}{sbl} \times sbl$								
	<i>SBL</i>	<i>sbl</i>	<i>SBl</i>	<i>sbL</i>	<i>Sbl</i>	<i>sBL</i>	<i>SbL</i>	<i>sBl</i>
	177	189	110	100	20	25	9	11
Expectation	190.4		105.7		15.6		8.7	
$\frac{SBL}{sbl} \times \frac{SBL}{sbl}$								
	<i>SBL</i>	<i>sbl</i>	<i>SBl</i>	<i>sbL</i>	<i>Sbl</i>	<i>sBL</i>	<i>SbL</i>	<i>sBl</i>
	366	361	215	216	50	57	21	26
Expectation	361.4		212.6		51.6		30.4	
$\frac{SBL}{sbl}$ selfed								
	<i>SBL</i>	<i>sbl</i>	<i>SBl</i>	<i>sbL</i>	<i>Sbl</i>	<i>sBL</i>	<i>SbL</i>	<i>sBl</i>
	1465	211	370	388	26	90	67	23
Expectation	1489.2	216.1	364.2	317.8	51.1	97.5	75.2	28.8
$\frac{BGL}{bgl} \times bgl$								
	<i>BGL</i>	<i>bgl</i>	<i>BGl</i>	<i>bgL</i>	<i>Bgl</i>	<i>bGL</i>	<i>BgL</i>	<i>bGl</i>
	289	264	12	11	136	155	5	1
Expectation	290.4		9.8		131.7		4.5	



$\varnothing bgl \times \frac{BGL}{bgl}$								
	BGL	bgl	BGl	bgl	Bgl	bGL	BgL	bGl
	511	495	8	12	296	278	4	3
Expectation	516.5		9.8		271.9		5.2	
<hr/>								
$\varnothing \frac{SBGL}{sbgl} \times sbgl$								
	SBGL	sbgl	SBGl	sbgl	Sbgl	sBGL	SBgl	sbGL
	86	85	3	3	11	9	44	42
Expectation	89		3		7.3		40.4	
	SBgL	sbGl	SbGL	sBGL	SbGL	sBgl	SbGl	sBgL
	2	—	—	1	2	2	—	—
Expectation	1.4		0.2		3.3		0.1	
<hr/>								
$\varnothing sbgl \times \frac{SBGL}{sbgl}$								
	SBGL	sbgl	SBGl	sbgl	Sbgl	sBGL	SBgl	sbGL
	362	359	4	9	49	57	211	207
Expectation	368.6		7		52.7		194	
	SBgL	sbGl	SbGL	sBGL	SbGL	sBgl	SbGl	sBgL
	4	2	—	—	21	26	1	—
Expectation	3.7		1		27.7		0.5	

In view of the various combinations involved in the parental constitutions, the results of self-fertilisations in the case of the four factors taken together could only be adequately set out at great length, which in proportion to the information they would add appears superfluous.

### Second Linkage Group.

Factors involved :

*F*, flat leaf in the absence of which the margin is crimped (*f*).

*Ch*, *sinensis*-shaped corolla and calyx many-toothed, usually 10 } in the absence of which { the corolla is *stellata* (*star*) and calyx 5-toothed (*ch*).

$\varnothing \frac{FCh}{fch} \times fch$  and reciprocal

<i>FCh</i>	<i>Fch</i>	<i>fCh</i>	<i>fch</i>
762	86	72	606

Linkage 8.6 : 1, or 10.4% of cross-overs.

$\frac{FCh}{fch}$  selfed

	<i>FCh</i>	<i>Fch</i>	<i>fCh</i>	<i>fch</i>
	1404	90	83	333
Expectation	1374	96.8	96.8	393.5

Calculated on linkage 8.6.

	$\frac{Fch}{fCh}$ selfed			
	<i>F Ch</i>	<i>F ch</i>	<i>f Ch</i>	<i>f ch</i>
	514	268	187	6
Expectation	489.2	241.7	241.7	2.6

Repulsion calculated as above.

## DESCRIPTION OF PLATES.

The drawings reproduced in these Plates were made by Mr C. H. Osterstock.

## PLATE X.

- Fig. 1. The deep red stems and leaf-backs formed in the absence of factor *L*.  
 Fig. 2. The light red corresponding parts showing inhibition of colour due to the presence of *L*.

## PLATE XI.

- Fig. 3. Semi-diagrammatic representation of epidermis of petal of a blue flower (*B*).  
 Fig. 4. Ditto of slaty flower (*b*).  
 Fig. 5. "Czar." A blue with coloured stigma, on light red stem (*BgL*).  
 Fig. 6. Slaty, coloured stigma, on light red stem (*bGL*).  
 Fig. 7. Blue, like Czar, but on dark stem (*Bgl*).  
 Fig. 8. Slaty, otherwise like Fig. 7 (*bgl*).  
 Fig. 9. Lee's crimp; *stellata*; single; genetically red. Calyx below.  
 Fig. 10. Lee's crimp; *sinensis*; single; magenta. Calyx below.  
 Fig. 11. Lee's crimp; *stellata*; single; P. Q. eye. Colour not determinable without breeding tests.

## PLATE XII.

- Fig. 12. "Etna." Red, red stigma, dark leaf (*Rgl*).  
 Fig. 13. A red, corresponding to Etna, with dark leaf, but having the stigma and gynoecium green (*RGL*).  
 Fig. 14. "Harlequin." *Sinensis* shape.  
 Fig. 15. " " *Stellata* shape.  
 Fig. 16. Mosaic flower: most of the petals *BRG*, the stripe being *bRg*. Eye P. Q.  
 Fig. 17. Corresponding uniform flower *BRG*. Eye P. Q.  
 Fig. 18. A flower uniformly of the same type as the stripe in Fig. 16, viz. *bRg*. Eye P. Q.  
 Fig. 19. Leaf of *sinensis*, P. Q. eye, crimped, ageing; showing great development of marginal points. The purple colour is exclusively on the lower surface.

## PLATE XIII.

- Fig. 20. Flat Palm.  
 Fig. 21. " Fern.  
 Fig. 22. " Oak.  
 Fig. 23. " Fern Oak.

## PLATE XIV.

- Fig. 24. Palm leaf, flat. Outline of margin divided to avoid imbrication.  
 Fig. 25. Oak leaf, flat. Outline of margin.  
 Fig. 26. Palm crimp, *stellata*. Outline of portion of margin magnified.  
 Fig. 27. " *sinensis*. " " " "

PLATE XV.

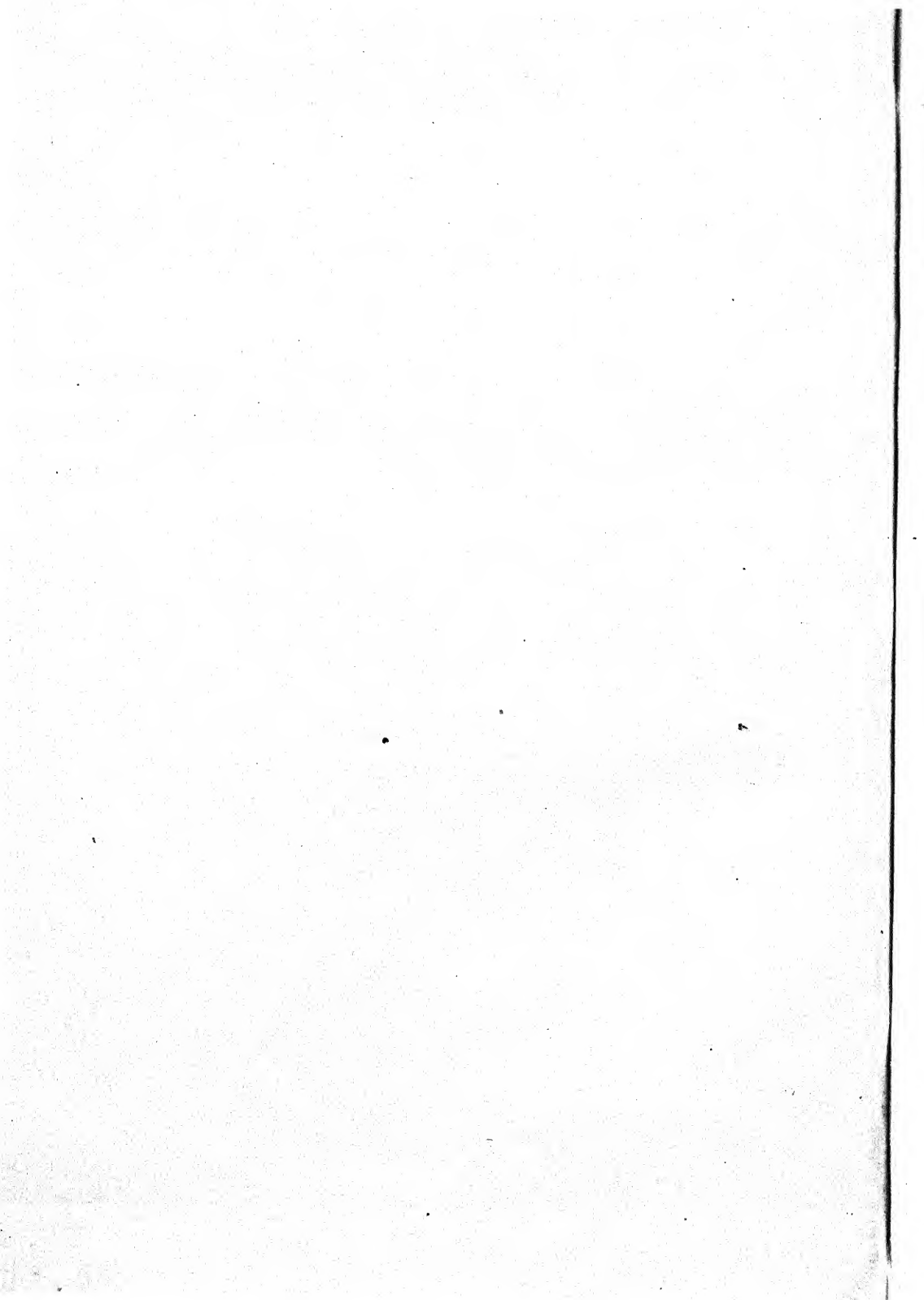
- Fig. 28. Lee's crimp, *stellata*. Leaf, calyx, and flower.  
 Fig. 29. „ „ *sinensis*. „ „ „ „  
 Fig. 30. Sutton's crimp, *sinensis*. The low degree of crimping.  
 Fig. 31. „ „ „ „ In combination with P. Q. eye, showing increase in crimping and malformation of flower as usual in this combination.

PLATE XVI.

- Fig. 32. Palm crimp  $\times$  Oak flat, with  $F_1$  and the four combinations in  $F_2$ .  
 Fig. 33. Flowers from oak leaved plant, *sinensis*, showing peculiar shapes common in this combination.  
 Fig. 34. Corresponding *stellata* flowers; petals narrow but not malformed.

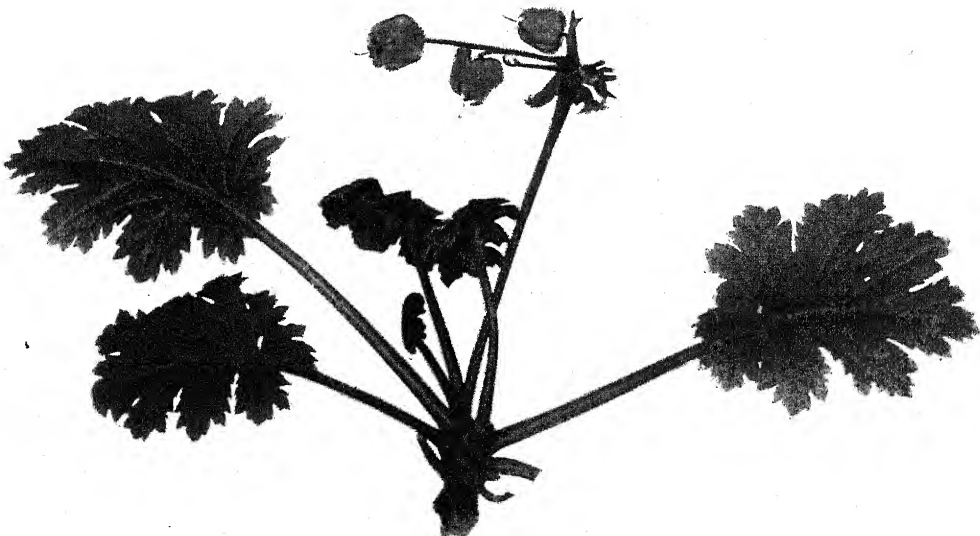
PLATE XVII.

- Fig. 35. Various Eyes.  
 A. White or "Queen Alexandra."  
 B. Normal on flat leaf.  
 C. "Primrose Queen" (P. Q.) eye on flat leaf.  
 D. Normal on Lee's crimp leaf: fringe here abnormally weak.  
 E. P. Q. on crimp leaf.  
 Fig. 36. Plant of crimp type combined with *sinensis* flowers as seen in July, with proliferation of crimping.



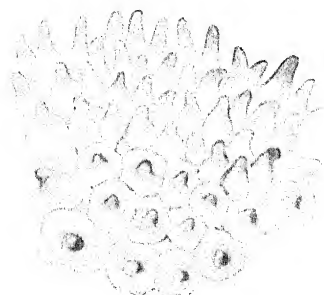


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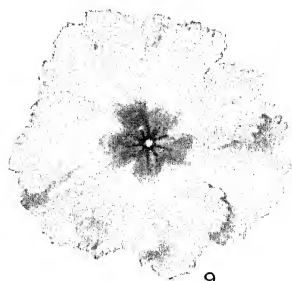


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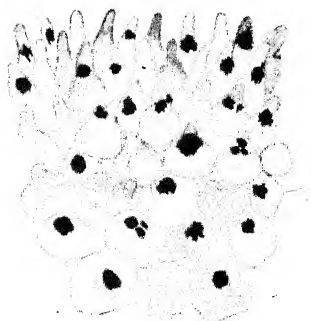




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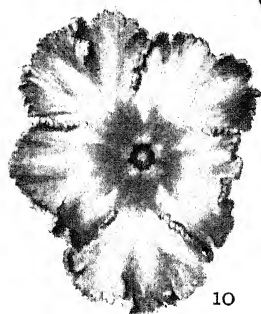
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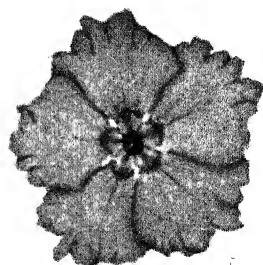
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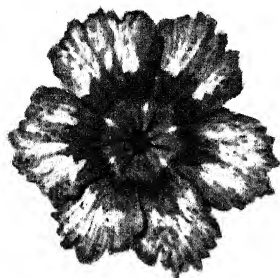
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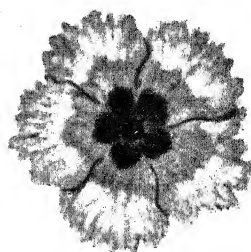
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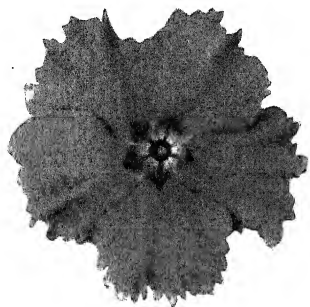
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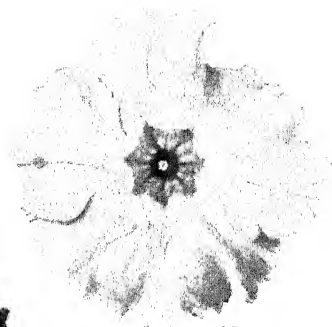
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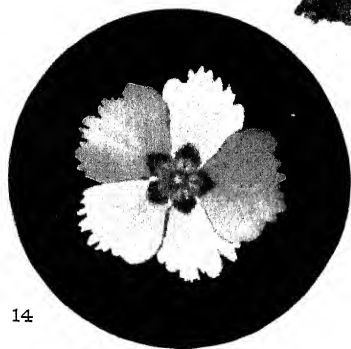
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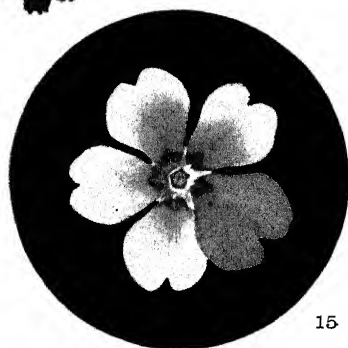
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14



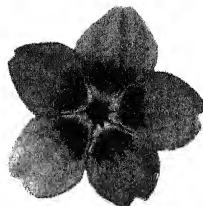
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16



17



18

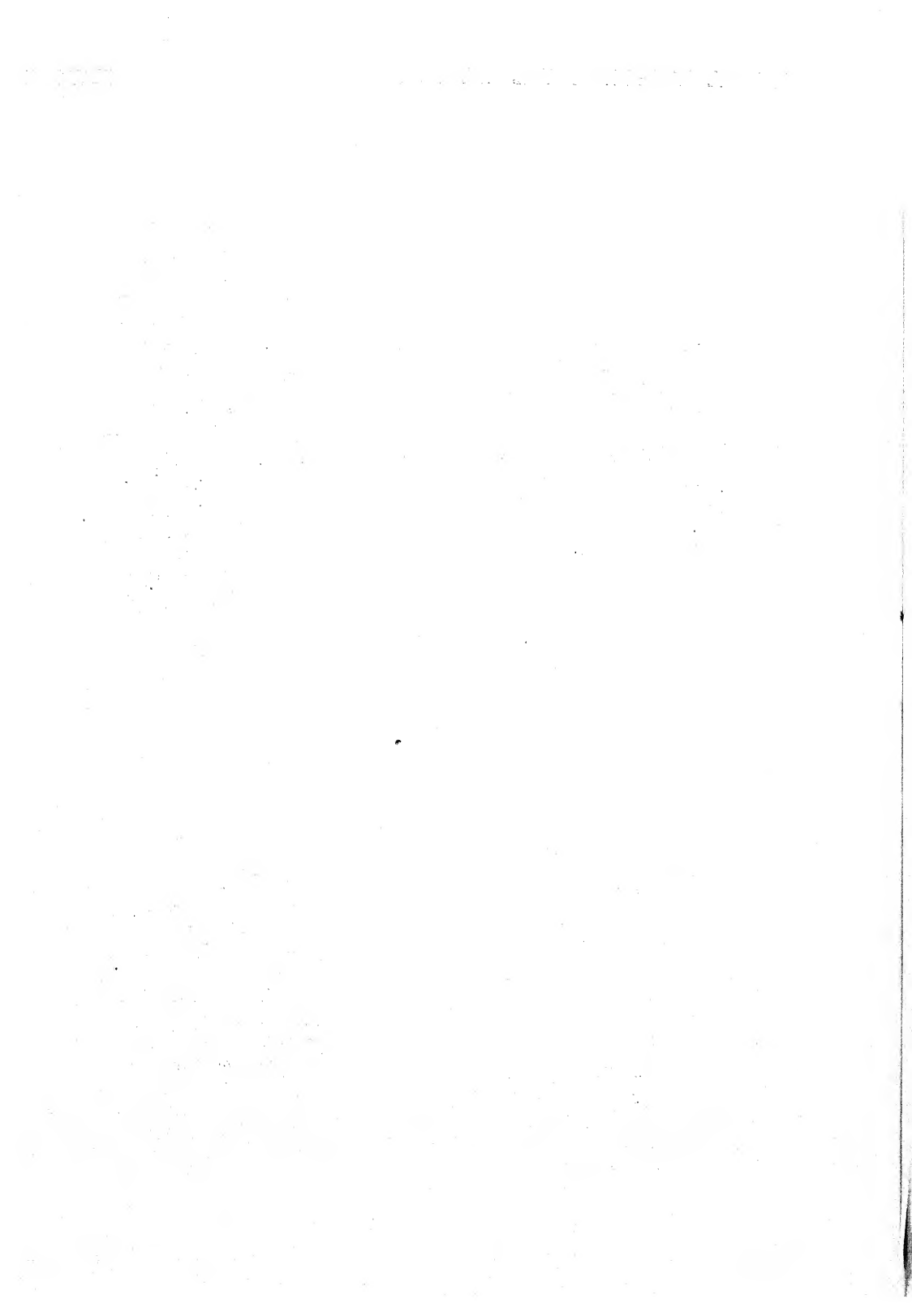




Fig. 20.



Fig. 21.

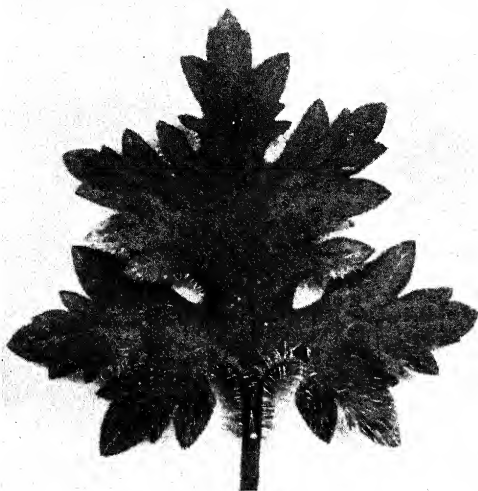


Fig. 22.

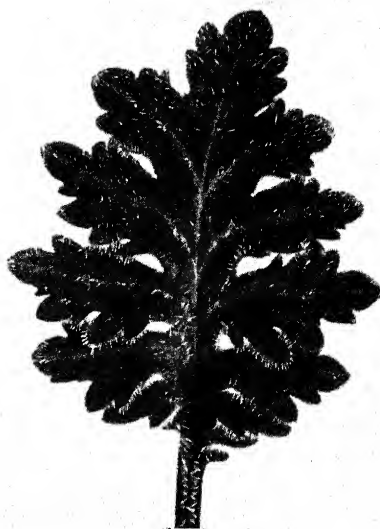
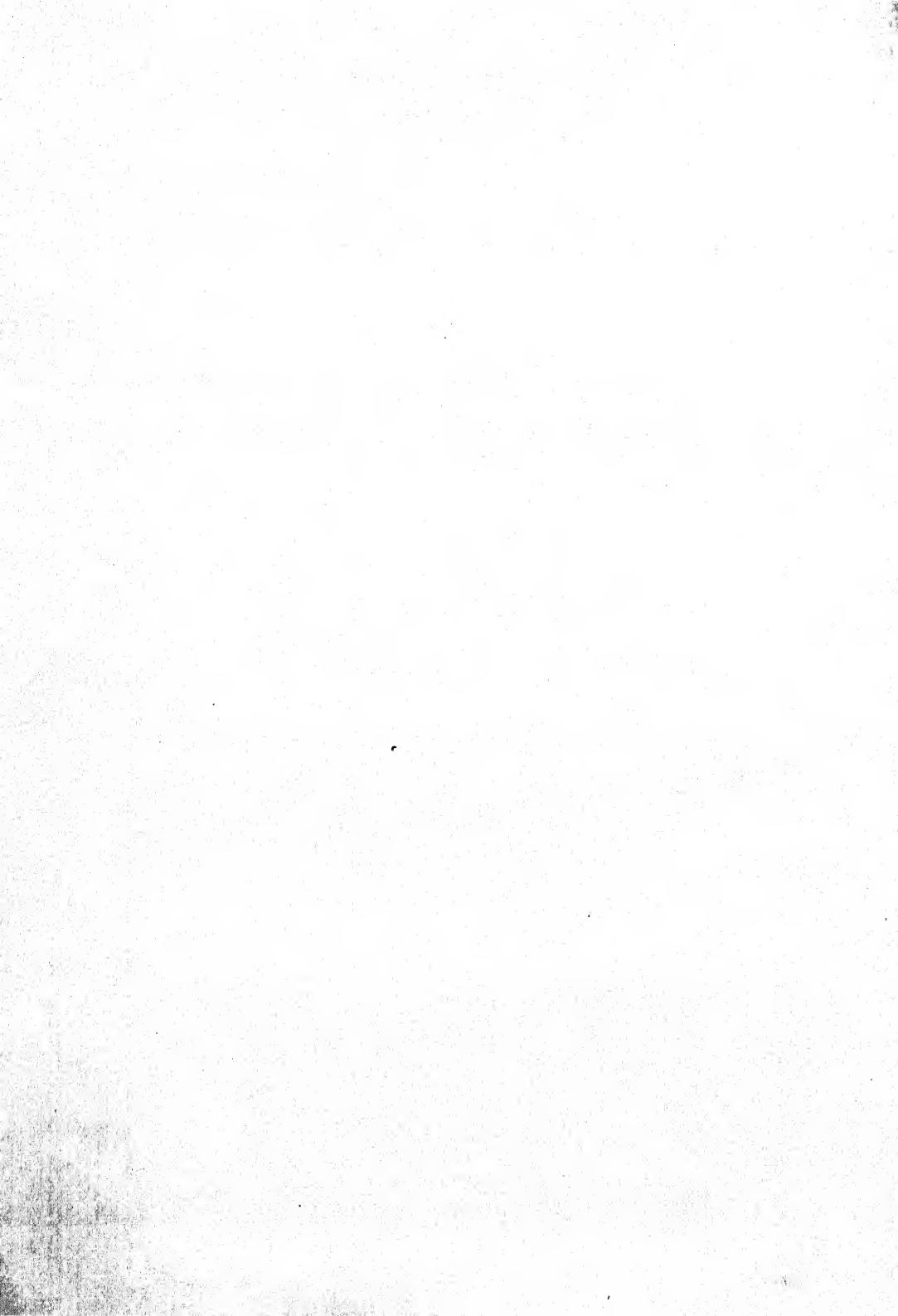


Fig. 23.



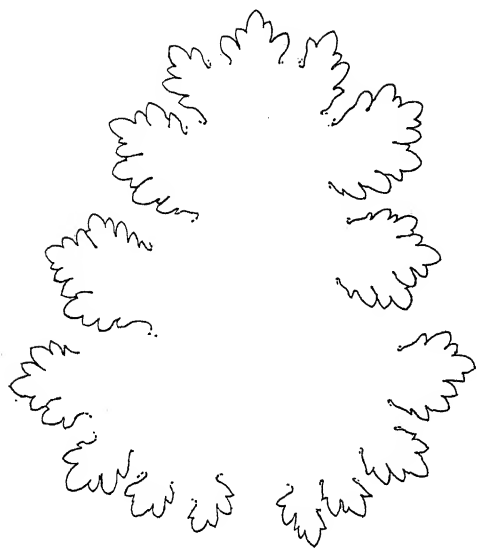


Fig. 24.

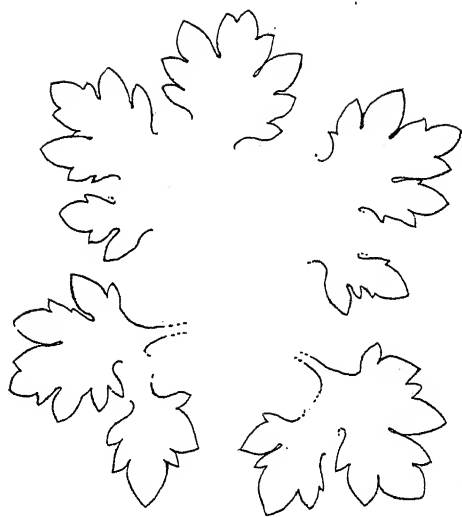


Fig. 25.



Fig. 26.

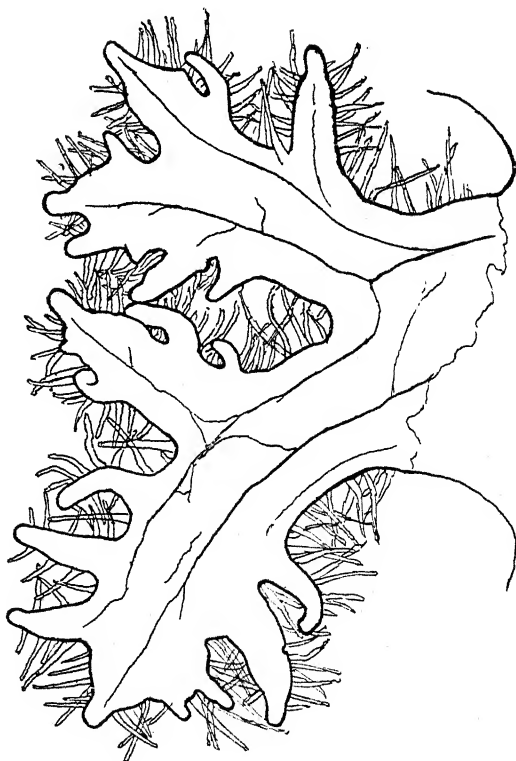
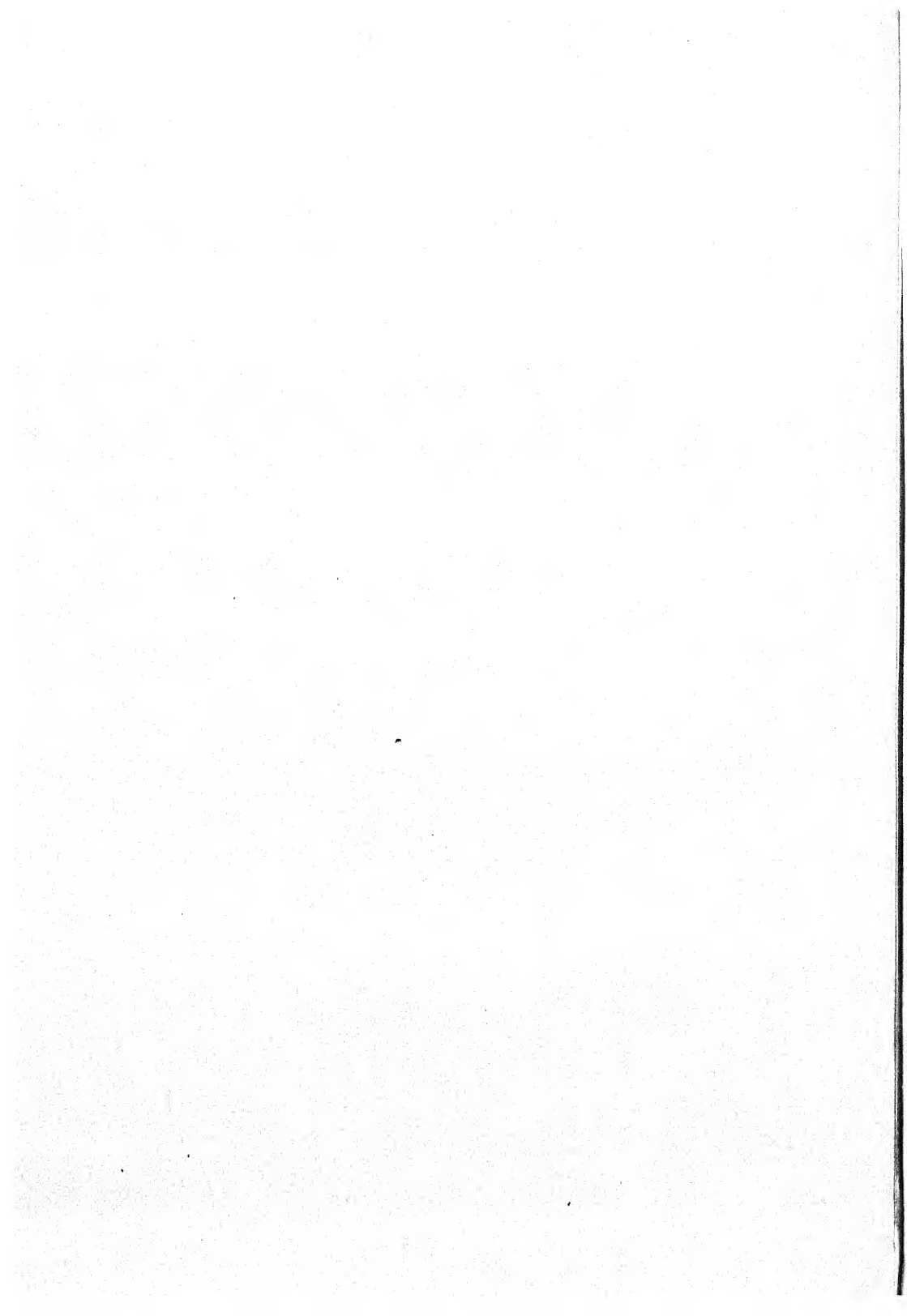
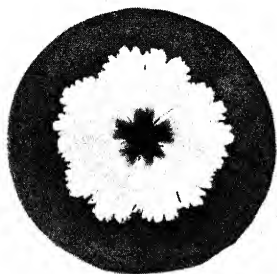
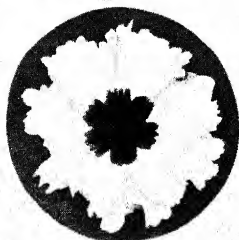


Fig. 27.





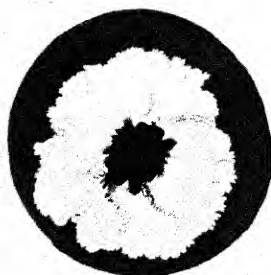
*Fig.28*



*Fig.30*



*Fig.30*



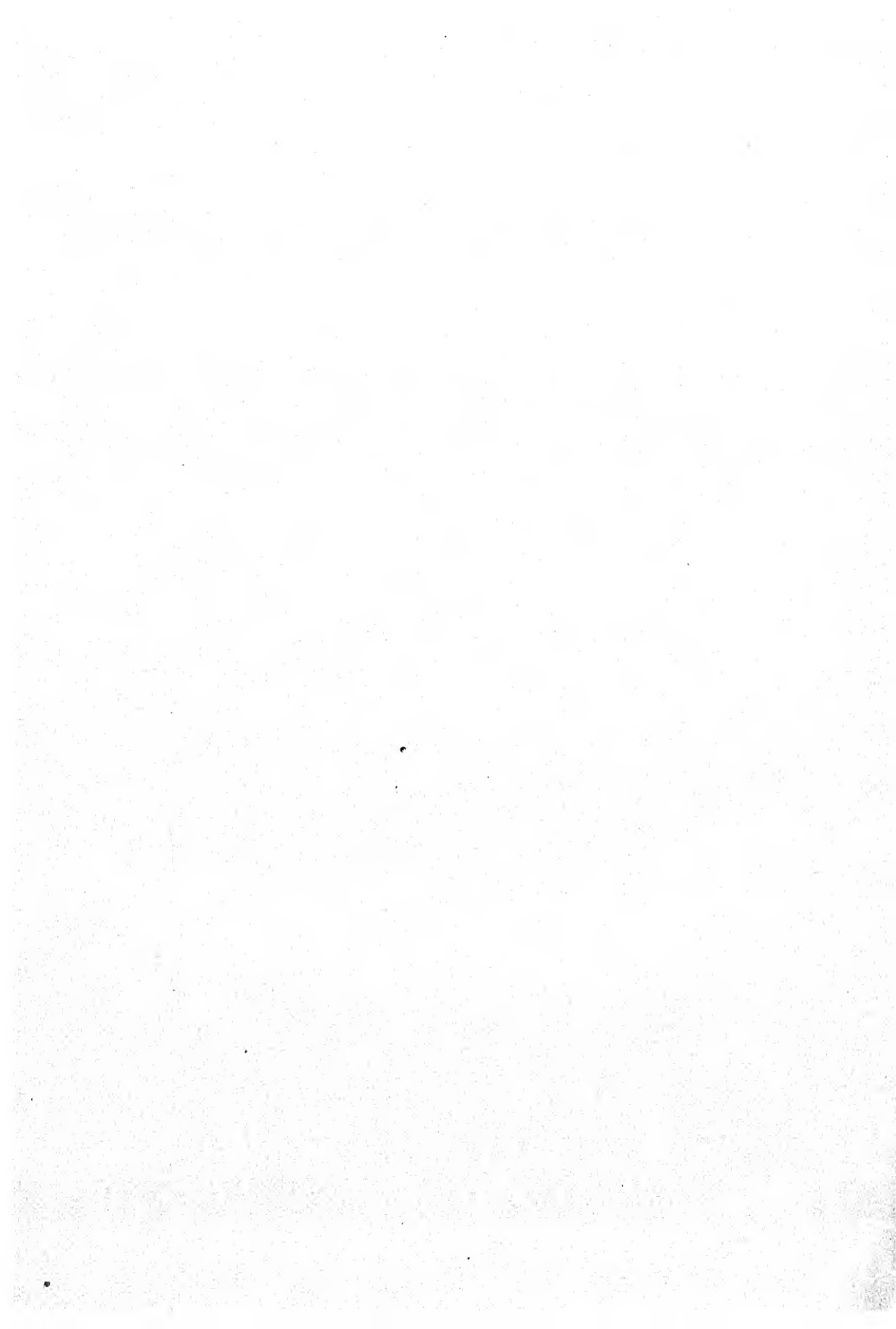
*Fig.29*



*Fig.31*



*Fig.31*



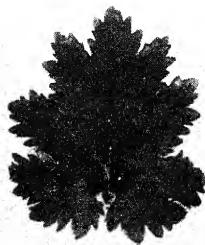




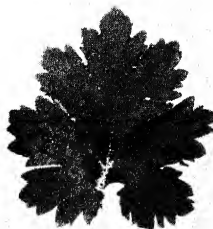
Palm crimp.



Oak flat.



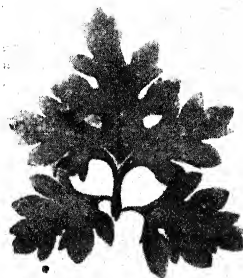
*F*<sub>1</sub> Palm flat.



*F*<sub>2</sub> Palm flat.



*F*<sub>2</sub> Palm crimp.



*F*<sub>2</sub> Oak flat.



*F*<sub>2</sub> Oak crimp.

Fig. 32.

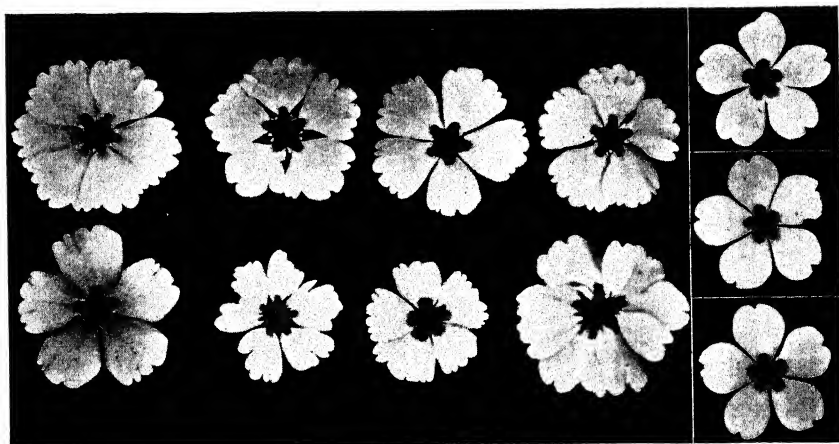
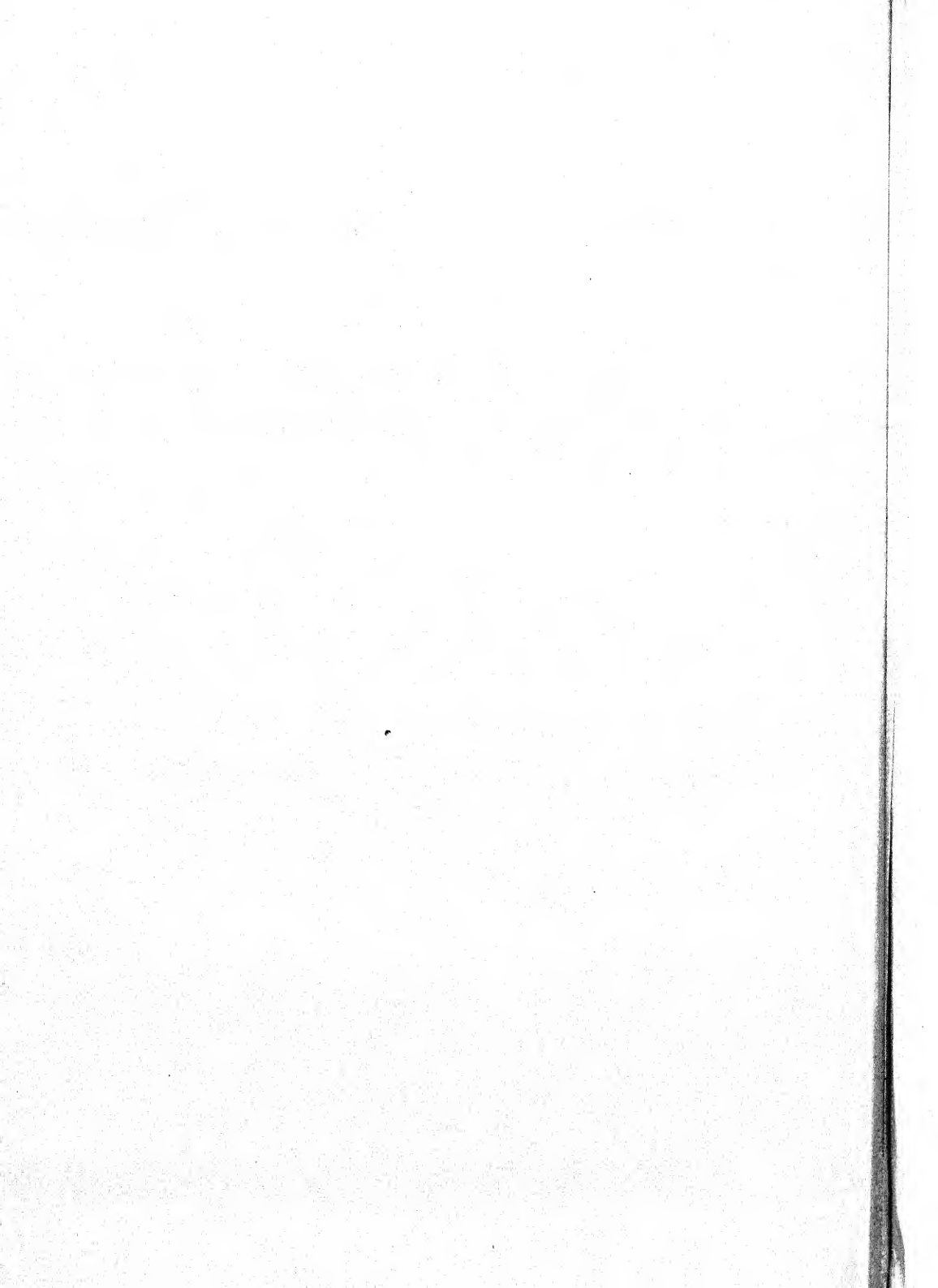
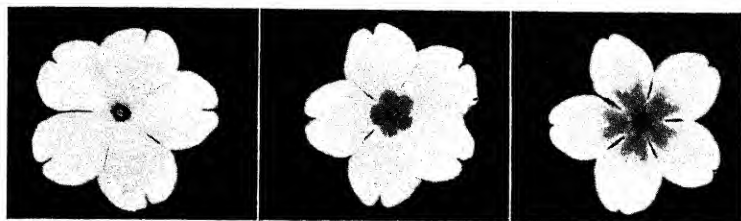


Fig. 33

Fig. 34

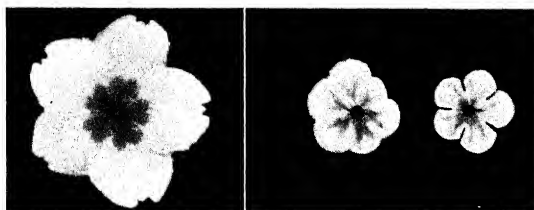




A. White Alex.

B. Normal on flat leaf.

C. Pr. Queen on flat leaf.



D. Normal on crimp leaf.

E. Pr. Queen on crimp leaf.

Fig. 35.

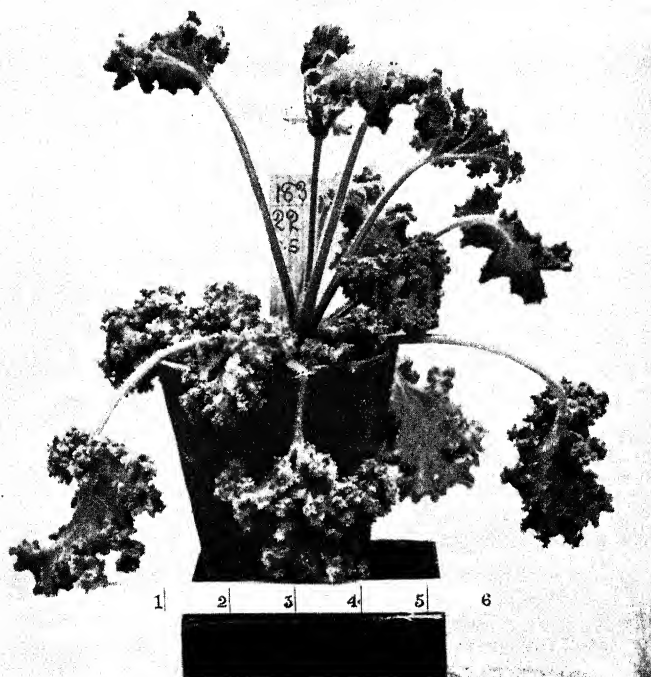


Fig. 36.



THE PROGENY, IN GENERATIONS  $F_{12}$  TO  $F_{17}$ , OF  
A CROSS BETWEEN A YELLOW-WRINKLED AND A  
GREEN-ROUND SEEDED PEA; A REPORT ON DATA  
AFFORDED BY EXPERIMENTS INITIATED BY THE  
LATE A. D. DARBISHIRE, M.A., IN 1905, AND  
CONDUCTED BY HIM UNTIL HIS DEATH IN 1915.

By G. UDN YULE, M.A., F.R.S.

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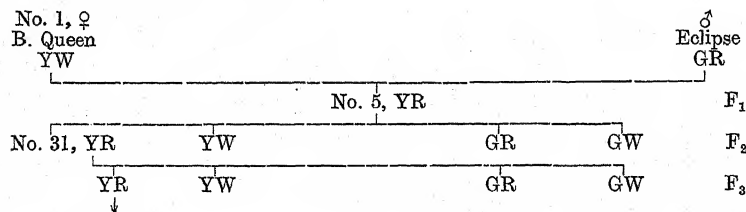
(With Eight Figures.)

THE investigation of which the results are discussed in the following pages forms virtually a continuation of the work begun by the late Mr A. D. Darbishire in 1905, the first results of which were described by him in a paper entitled "An experimental estimation of the theory of ancestral contributions in heredity<sup>1</sup>." After his death in December 1915, Miss Darbishire was anxious to maintain the experiments, at least until the data already accumulated could be in some degree reduced and their indications estimated, so that the value of the work already done might not be lost. With the assistance of grants from the Royal Society, for which Miss Darbishire here desires to return her most grateful thanks, this was rendered possible. Thanks are also due to the late Mr F. J. Bridgeman, then demonstrator at University College, London, who helped in the sowing and harvesting of 1916, but especially to Mr Frank Sherlock, Senior Assistant in the Dept. of Zoology and Comparative Anatomy, University Museum, Oxford. Mr Sherlock not only acted as assistant to Mr Darbishire during the latter years of the experiments in the sowing, harvesting, and recording of the crops, but since Mr Darbishire's death has been almost solely responsible for the work. It is difficult to speak too highly of the time and trouble he has very generously given for many years. Since, at the request of Miss Darbishire, I undertook the reduction of the data, I can add my tribute to the care and accuracy with which all the records appear to have been made, care and accuracy which have greatly facilitated my own work.

<sup>1</sup> *Proc. Roy. Soc. B.* Vol. LXXXI. 1908, p. 61.

## I. THE NATURE OF THE EXPERIMENTS.

In the spring of 1905 Mr Darbishire obtained from Mr C. C. Hurst<sup>1</sup> some seeds, the cotyledons of which belonged to the  $F_3$  generation of a cross which he had made between the two peas British Queen and Eclipse. All the seeds had been borne on a single plant, the pedigree of which he gives as follows:



The yellow-round seeds of the sample (i.e. the YR-cotyledoned plants of the  $F_3$  generation) were sown in the spring of 1905, and the  $F_4$  harvested in the autumn of that year. Of this  $F_4$  generation only the YR were, as before, sown in the spring of 1906, and  $F_5$  was harvested in the autumn of that year.

It appears from this description that at first, while only YR seeds were sown<sup>2</sup>, these were taken miscellaneously from the entire harvest. Later only YR seeds were sown from plants bearing all four types of seed (dihybrid plants): ten seeds were sown from each of some 30 or more dihybrid plants; these ten were sown together and the plot given a number, e.g. G. 1, identifying the parent plant, each plant in the plot bearing also a subsidiary number, G. 1. 1, G. 1. 2, ... G. 1. 10. Ten seeds were again saved from one dihybrid plant of each plot, and so on.

The generations available for the present discussion are the following: the generation stated being that of the seeds harvested, e.g. the seeds on the plants G, harvested in 1913, were  $F_{12}$ , the plants themselves being  $F_{11}$ . The experiments were closed after 1918 owing to serious failure of the following crops:

Year	Letter	F
1913	G	12
1914	H	13
1915	J	14
1916	K	15
1917	L	16
1918	M	17

In the opening sentence of the paper cited, Mr Darbishire states that "the experiments described in the following pages were undertaken with the object of finding out if the proportions in which characters segregate

<sup>1</sup> *Proc. Roy. Soc. B.* Vol. LXXXI. 1908, p. 61.

<sup>2</sup> I have not been able to follow the earlier sowing records.

in the  $F_2$  generation are affected by the distribution of those characters over the parentage and the ancestry of the forms crossed," and the experiments, I take it, were continued in the same spirit of pure scientific scepticism to see whether, if the work were continued on the same lines, the fact that the ancestry were "YR" without a break would have any influence on the Mendelian proportions or whether these would remain stable. "I consider the Mendelian principles to be still *sub judice*," he wrote<sup>1</sup> in the spring of 1915, "and they are so attractive by reason of their simplicity that they need to be under a very stern judge."

The data were never reduced during the course of the experiments. I have some recollection of a conversation with Mr Darbishire in which he took the view that such a course would be undesirable, as temporary results might tend to bias his judgment and he wished to be able to discuss the data, which he hoped to maintain as far as  $F_{20}$ , as a whole. The disadvantages of the course adopted—which possibly he might have seen reason to alter had he again been able to give the experiments his personal attention—are now obvious, as it has rendered it impossible to test, by further selfing or by crossing, the real genetic constitution of plants bearing apparently abnormal proportions of seeds. The data must be taken as they stand.

I have given the report in the following order, representing broadly the order in which the work was done:

II. The proportions of different types of plant (dihybrids, monohybrids for shape, monohybrids for colour, and pure dominants) grown from the YR seeds. (Pp. 262–271.)

III. The proportions of seeds on each type of plant in each crop as a whole. (Pp. 271–278.)

IV. The proportions of seeds on individual plants of each type. (Pp. 278–293.)

V. Markedly aberrant plants, their families and descendants: classification of seeds and of plants by lines of descent. (Pp. 293–323.)

VI. Proportions of seeds of each type, and of the possible combinations, in the pods of dihybrid plants. (Pp. 323–331.)

That the discussion must have suffered, and suffered greatly, from the impossibility of talking matters over with the originator of the experiments and from ignorance of the precise questions in his mind is only too obvious. I cannot say how much, again and again, the futile desire for the renewal of our interrupted conversations has arisen in the course of the work. The labour given to it I dedicate gladly to his

<sup>1</sup> I cite from Miss Darbishire's preface to *An Introduction to a Biology*, by A. D. Darbishire. Cassell, 1917.

memory, hoping that he will pardon the inevitable failure of a statistician who is not a biologist to make of it all that he would have wished.

It will be convenient to give here a summary of the principal results. Brief references are given to tables and figures illustrative of the points mentioned, but these must, of course, be taken in conjunction with the discussions in the text:

(1) The YR seeds from dihybrid plants do not yield plants of the four possible types (dihybrids, monohybrids for shape, monohybrids for colour and pure dominants) in the expected proportions 4 : 2 : 2 : 1 or 445 : 222 : 222 : 111 per 1000, but in the average proportions 405 : 221 : 234 : 140 (Table I, p. 264). There is a marked excess of pure dominants and a deficiency of dihybrids as compared with the simple Mendelian theory. The variations in the observed proportions from crop to crop from the general average are within the limits of fluctuations of sampling.

(2) The proportions on dihybrids of seeds classified as YR's, YW's, GR's and GW's fluctuate from year to year to an extent exceeding the limits of fluctuations of sampling (Table VI (A), p. 273). Crops *J* and *K* agree well with Mendelian expectation (9 : 3 : 3 : 1), but Crops *G*, *L* and *M*, especially the last, diverge. On the average of the six crops the proportions given are 5658 : 1888 : 1872 : 583 per 10000, instead of 5625 : 1875 : 1875 : 625. There is a large deficiency of GW seeds, mainly compensated by an excess of YR's. These results are fairly parallel to those published in the *Second Report of the Evolution Committee of the Royal Society*.

For monohybrid plants the proportions of seeds seem fairly normal (Table VI (B), (C), pp. 273, 274), though monohybrids for colour tend to a slight deficiency of Yellows.

(3) The frequency distribution showing numbers of dihybrid plants (with 100 seeds or more) giving each percentage of YR seeds, diverges largely from expectation (Fig. 1, p. 259). There is no mode at or near the expected proportion 56.25 per cent. (9/16) but a marked peak at 58 per cent. and a suggestion of a peak at 51-52 per cent. The distribution looks, in fact, compound. The extracted distribution for very large plants with 200 seeds or more (Fig. 7, p. 261) looks even more markedly compound, with modes at about the same percentages. Trial shows that the distribution for all plants with 100 seeds or more can be closely represented by a compound distribution (Fig. 8, p. 261), but there is a serious difficulty in so regarding it, in that the standard deviations of the components would be very much lower than the standard deviations of sampling (p. 289).



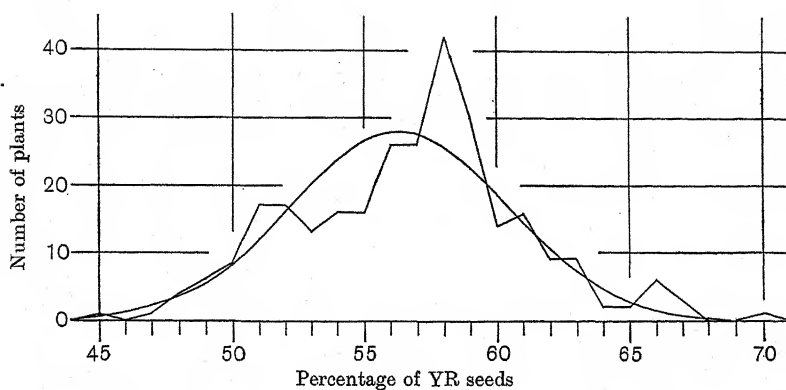


Fig. 1.

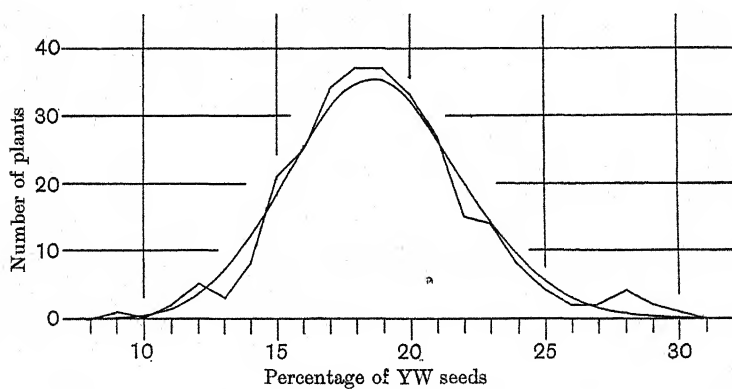


Fig. 2

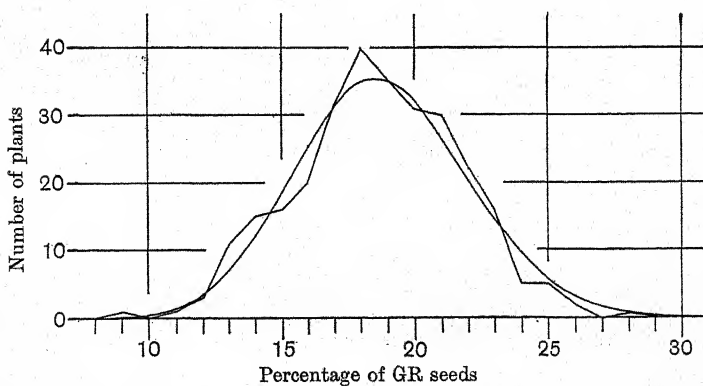


Fig. 3

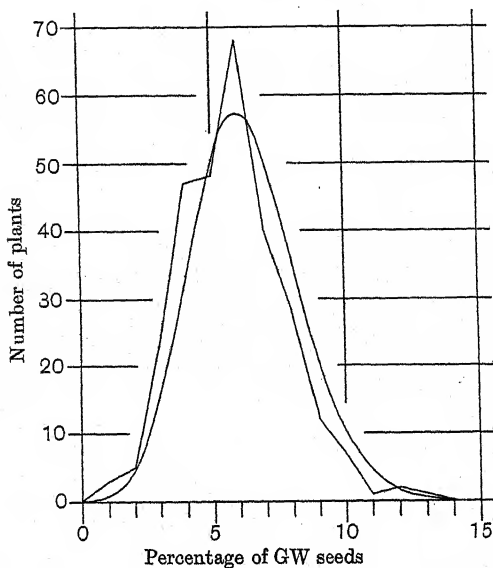


Fig. 4

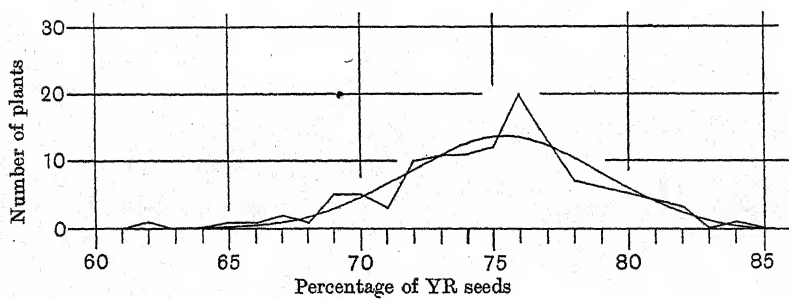


Fig. 5

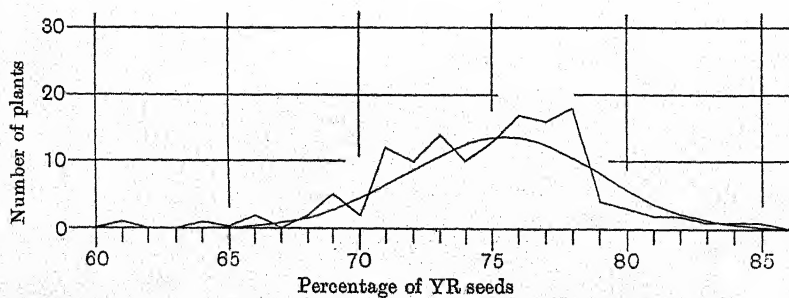


Fig. 6

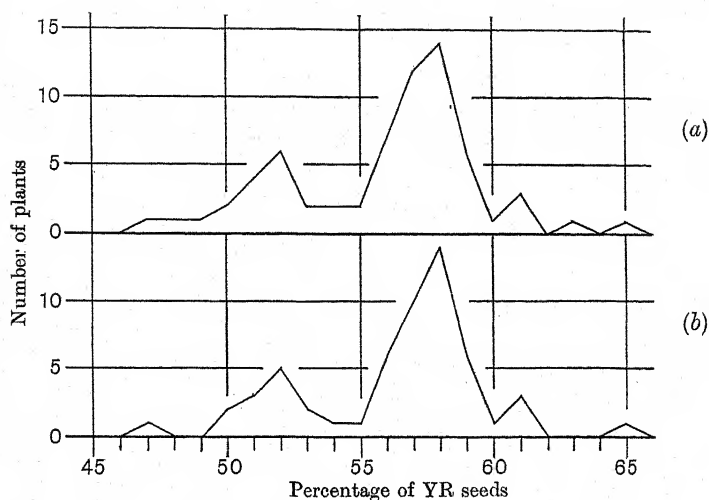


Fig. 7

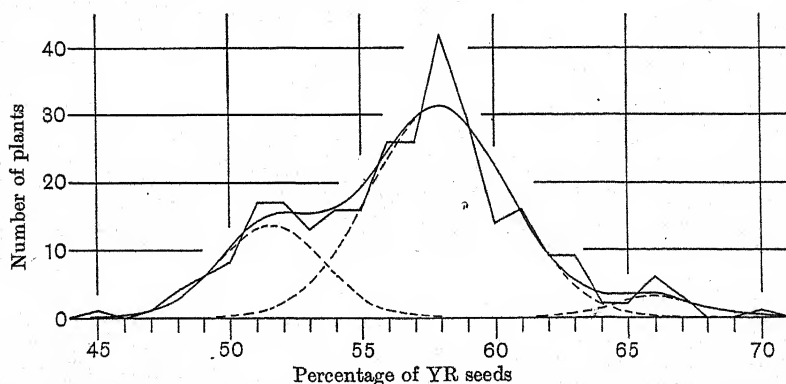


Fig. 8

The frequency distributions for monohybrid plants are less irregular (Figs. 5 and 6, p. 260), but that for hybrids for colour is also apparently abnormal (p. 292).

(4) An examination of individual dihybrid plants by the proportions of YR : YW : GR : GW seeds shows an excess of markedly divergent plants over the numbers expected on the theory of random sampling (Table XIII, p. 295). There seems to be some tendency for such abnormal plants to cluster in families (p. 299). An examination of the seeds on dihybrid plants and of the plants grown from YR seeds grouped by lines of descent (Table XVI, p. 307) shows that, while a number of such lines give average proportions approximately in accordance with Mendelian

expectation, others are highly aberrant. The various lines cannot be regarded as forming a homogeneous group.

For the monohybrids on dihybrid lines (Tables XXII, XXIII, pp. 318, 319) there is little evidence of heterogeneity. The lines of monohybrids for shape are *more* self-consistent than would be expected on the theory of random sampling. Monohybrids for colour, however, give one line that is highly abnormal, showing 81.1 per cent. of yellows on 523 seeds (pp. 319, 320).

(5) The deficiency of GW seeds, the excess of YR seeds, the deficiency of dihybrid plants and the excess of pure dominants given by the totals of the six crops, seem to be *mainly* due to particular lines forming together about half the whole number (pp. 310-312).

(6) It is not possible to account for the variations from crop to crop in the proportions of seeds on dihybrid plants by variations in the proportionate contributions of different lines to the crop. The variations appear to affect all lines (Table XXIV (2), p. 321).

(7) A sample investigation on the pods of dihybrid plants of Crop G showed good agreement on the whole, with the expectation of random sampling, in the numbers of seeds of each type in pods of each size (Table XXVI, p. 326). The frequencies of the possible combinations of YR, YW, GR and GW seeds in five-seeded pods (the most frequent size) gave quite good agreement with expectation (Table XXVII, p. 328), and (with the possible exception of one or two combinations) the same was true of the five-seeded pods of Crops J and K (Table XXVIII, p. 330). The abnormalities noted above do not appear appreciably to affect the distributions in the pods.

No explanation is offered of the remarkable divergences from the expectation based on simple Mendelian theory; they remain a puzzle. But it seems clear that that theory is inadequate completely to explain all the facts. The mechanism at work appears to be more complex than is commonly postulated.

## II. THE PROPORTIONS OF DIFFERENT TYPES OF PLANT FROM THE YR SEEDS.

Since yellowness and roundness are dominant, the types of yellow-round seeds borne by the self-fertilised dihybrid will be given by expanding

$$(Y + G)^2 (R + W)^2$$

and picking out the terms containing Y and R. They are as follows:

- |           |   |   |
|-----------|---|---|
| A. 4YG.RW |   | giving plants bearing all four types of seed. |
| B. 2YY.RW | “ | “ YR + YW seeds.                              |
| C. 2YG.RR | “ | “ YR + GR “                                   |
| D. 1YY.RR | “ | “ YR only.                                    |

In the future I shall use the letters *A*, *B*, *C*, *D*, as above, to denote these four types of plant. *A*'s are the dihybrids bearing all four types of seed in the ratio 9YR : 3YW : 3GR : 1GW; *B*'s are the hybrids for shape, bearing rounds to wrinkleds in the ratio 3 : 1; *C*'s are the hybrids for colour bearing yellows to greens in the ratio 3 : 1, and *D*'s are the pure dominants bearing nothing but yellow-round seeds. In each generation *A*'s, *B*'s, *C*'s and *D*'s should appear, if the simple Mendelian theory is correct, in the proportions 4 : 2 : 2 : 1.

In Table I are shown the actual numbers of *A*, *B*, *C* and *D* plants in each of the six crops, together with the expected numbers. It will be seen that there are some very considerable divergences from expectation, only Crop *M* giving a really good agreement. If the results for all the crops are added together, as in the lowest section of the table, the disagreement is emphasised, showing that the divergences in the various years are in some degree in consonance, there being a general tendency to a deficiency of *A*'s and an excess of *D*'s. In the last column of the table are given the values of *P*, the probability that a fit as bad as or worse than that observed might arise on random sampling from a universe containing *A*, *B*, *C* and *D* plants in the theoretical proportions 4 : 2 : 2 : 1, calculated in the now well-known way by Prof. Pearson's  $\chi^2$  method<sup>1</sup>. For the first crop, *G*, the fit is exceedingly bad, the chance of getting such a divergence on random sampling being less than 1 in 2000; such a divergence is almost certainly evidence that something besides mere chance is at work. For Crop *H*, *P* is also small; we would only expect to get such a divergence as that observed about three times in 200 trials, and it may be noted that the main divergences between observation and theory are similar to those observed in the preceding generation, viz. excess of *D*'s and deficiency of *A*'s. In the following generations the values of *P* are much larger, but if the generations *J* to *M* are aggregated we once more find the same feature—excess of *D*'s and deficiency of *A*'s—though not quite so marked. For the six generations pooled together *P* is only .00007, so that we would only expect to find so great a divergence on random sampling about once in 15,000 trials. We can hardly write down such a series of results as due to nothing more than fluctuations of sampling.

We may put the question in another way by asking what is the

<sup>1</sup> *Tables for Statisticians and Biometricians*: Cambridge University Press. Each difference of the observed from the expected frequency is squared, the square divided by the expected frequency, and the sum of the quotients gives  $\chi^2$ . Table XII in the above volume is then entered in the column in which *n'* is the number of groups (four in the present case) if comparison is being made with frequencies given *a priori*; otherwise *n'* must be taken as the number of algebraically independent differences increased by unity.

probability of obtaining a collection of  $\chi^2$ 's as bad as or worse than that shown by the six crops. The answer is given by summing the  $\chi^2$ 's, giving a total of 44.25, and entering the tables with  $n' = 3 \times 6 + 1$  or 19<sup>1</sup>. This gives  $P = .0012$ . Again, this is a very low value, though not nearly so low as the value obtained by pooling the six crops together. There is clearly a certain agreement in sign between the divergences of the several crops from expectation, which renders the probability of the pool much lower than that of the collection of  $\chi^2$ 's observed.

We can apply yet a further test to see how far the successive crops are consistent in the directions of their divergence from the expected Mendelian proportions. It is an obvious question whether the divergences of each generation from the average of the six may be regarded as random. Treating Table I as a contingency table, i.e. calculating the "expected" number of plants in each crop not from the 9 : 3 : 3 : 1 ratio but from the proportions given by the totals at the foot of the table, I find  $\chi^2$  to be 21.29;  $n'$  in this case is to be taken<sup>2</sup> as 16 (i.e.  $3 \times 5 + 1$ )

TABLE I.

*Showing the numbers of plants of each type in each crop compared with the numbers expected on the proportions 4 : 2 : 2 : 1.*

						A = dihybrid plants bearing YR + YW + GR + GW seeds B = monohybrid plants bearing YR + YW C = " " " " YR + GR D = pure dominants " " YR	
						Probability P of a fit as bad or worse arising on random sampling and $\chi^2$	
		TYPE OF PLANT					
Crop		A	B	C	D	Total	
G	Obs.	129	99	85	58	371	P .0004
	The.	164.9	82.4	82.4	41.2	—	$\chi^2$ 18.09
H	Obs.	134	73	72	55	334	P .018
	The.	148.4	74.2	74.2	37.1	—	$\chi^2$ 10.13
J	Obs.	135	58	84	34	311	P .17
	The.	138.2	69.1	69.1	34.6	—	$\chi^2$ 5.07
K	Obs.	126	63	70	47	306	P .11
	The.	136	68	68	34	—	$\chi^2$ 6.14
L	Obs.	117	73	67	38	295	P .37
	The.	131.1	65.6	65.6	32.8	—	$\chi^2$ 3.20
M	Obs.	134	57	70	35	296	P .66
	The.	131.6	65.8	65.8	32.9	—	$\chi^2$ 1.62
G	Obs.	775	423	448	267	1913	P .00007
to M	The.	850.2	425.1	425.1	212.6	—	$\chi^2$ 21.81

<sup>1</sup> Yule, *Introduction to the Theory of Statistics*, 6th Edition, Supplement III.

<sup>2</sup> R. A. Fisher, "On the Interpretation of  $\chi^2$  from Contingency Tables, and the Calculation of P," *Journal of the Royal Statistical Society*, Vol. LXXXV. 1922, p. 87; and Yule, "On the Application of the  $\chi^2$  Method to Association and Contingency Tables," *Ibid.*, or reference of the preceding note.

and  $P$  is 0.128. We might therefore expect a worse result on random sampling from a universe in which the true proportions were those given by the total of the crops (viz. 405 : 221 : 234 : 140) about once in eight trials. The numbers found cannot be regarded as random samples from the expected Mendelian proportions of 4 : 2 : 2 : 1 or 445 : 222 : 222 : 111, but can be regarded as random samples from proportions of

$$405 : 221 : 234 : 140.$$

If we wish further to judge how far each *individual* crop can be regarded as a random sample from the total, this can be done by condensing the table into a two-row contingency table in which the particular crop under consideration is one row and the aggregate of the remaining crops is the second row, working out  $\chi^2$  again for this table and entering the tables with  $n'$  equal to 4. The results are as follows:

Crop	$\chi^2$	$P$
<i>G</i>	9.10	.028
<i>H</i>	2.44	.493
<i>J</i>	7.28	.065
<i>K</i>	0.99	.803
<i>L</i>	1.53	.680
<i>M</i>	4.41	.225

The value of  $P$  is lowest for the first crop: a worse result would only be expected some three times in 100 trials in random sampling. Crops *H*, *K*, *L* and *M* are all in very fair agreement with the average.

I think it may be definitely concluded that the proportions of *A*, *B*, *C* and *D* plants given by these generations differ significantly from the expected Mendelian proportions and more nearly resemble expected proportions of 405 : 221 : 234 : 140.

In what direction are we to look for an explanation of this divergence? In the first place some investigation is necessary to see whether it is not due to possible errors of classification of the plants. If a *B* plant, for example, has very few seeds it may, as a mere matter of chance, show no YW seeds and be classified as a *D*. If an *A* plant has very few seeds it may, as a mere matter of chance, show no GR's or GW's and be classified as a *B*; or no YW's or GW's and be classified as a *C*; or even nothing but YR's and be written down as a *D*. Are such transfers likely to be of any importance?

If an *A* plant bears  $n$  seeds, the chance of these being all YR's and the plant being consequently classed as a *D* is  $(9/16)^n$ .

As regards the possibility of its being classed as a *B* (or *C*) we must settle how we shall place it if it bears only, say, YW seeds. The point is of no practical consequence, as no such plants to the best of my memory

actually occur; but suppose we agree that such plants shall be written down as *B*'s. Then the chance that the *A* plant will bear only YR or YW seeds is  $(3/4)^n$ ; but this includes the case of its bearing YR seeds alone, when it would be classed as a *D*, the chance of this event being  $(9/16)^n$ . The chance of the plant being classed as a *B* is therefore  $(3/4)^n - (9/16)^n$ . The chance of its being classed as a *C* is, of course, the same.

The chance of a *B*, or a *C*, being classed as a *D* is evidently  $(3/4)^n$ .

But what we want to know is the chance that a plant classed as a *D* is really an *A*, or a *B*, and so on. To solve this problem we must assume what is the true distribution of plants, and we will naturally assume the Mendelian distribution of 4 : 2 : 2 : 1. The distribution of *B*'s, *C*'s and *D*'s in the actual classification, if all the plants have  $n$  seeds each, is then:

	Classed as <i>B</i>	Classed as <i>C</i>	Classed as <i>D</i>
True <i>A</i>	$\frac{1}{4} \{(\frac{3}{4})^n - (\frac{9}{16})^n\}$	$\frac{1}{4} \{(\frac{3}{4})^n - (\frac{9}{16})^n\}$	$\frac{1}{4} (\frac{9}{16})^n$
True <i>B</i>	$\frac{2}{3} \{1 - (\frac{3}{4})^n\}$	—	$\frac{2}{3} (\frac{3}{4})^n$
True <i>C</i>	—	$\frac{2}{3} \{1 - (\frac{3}{4})^n\}$	$\frac{2}{3} (\frac{3}{4})^n$
True <i>D</i>	—	—	$\frac{1}{3}$

From this table, taking each column in turn, we can calculate, for any given number of seeds, the proportion of plants classed as *B*'s that are probably *A*'s; the proportion classed as *D*'s that are probably *A*'s, or either *B*'s or *C*'s, and so on. The results of the calculation for plants with 5 to 20 seeds are given in Table II. When the number of seeds exceeds 20 any chances of transfer become very small.

The actual numbers of plants with 5 to 20 seeds each in the totality of the six generations are given in Table III. Applying Table II to the data I find the following as the probable transfers:

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
From <i>D</i> to <i>A</i>	+0.16	—	—	-0.16
From <i>D</i> to <i>B</i> and <i>C</i>	—	+1.08	+1.08	-2.16
From <i>B</i> to <i>A</i>	+1.80	-1.80	—	—
From <i>C</i> to <i>A</i>	+2.16	—	-2.16	—
Total	+4.12	-0.72	-1.08	-2.32

The plants transferred from *D* to *B* or *C* have been divided equally between the two. It will be seen that the net effect is quite small. Transfers owing to misclassification of plants with few seeds can only permit of an addition by way of correction of some four plants to the *A*'s, a deduction of about one plant apiece from the *B*'s and the *C*'s, and a deduction of two or three plants from the *D*'s. We have to account for an excess, not of two or three, but of 55 plants in the *D*'s (Table I), and a deficiency not of some four plants in the *A*'s, but of 75. It seems clear that the point we have raised is of little practical importance in the present



case<sup>1</sup>, and can do little towards accounting for the discrepancy between Mendelian expectation and the numbers of plants of the different types observed as shown in Table I.

TABLE II.

*Showing, for a given number of seeds on the plant from 5 to 20, the proportion of plants classed as D's that are probably A's, B's or C's; and the proportion of plants classed as B or C that are probably A's.*

No. of seeds	Proportion of D's that are		Proportion of B's or C's that are A's
	A	B or C	
5	.1036	.4365	.3219
6	.0689	.3872	.2625
7	.0444	.3326	.2107
8	.0278	.2780	.1681
9	.0171	.2270	.1306
10	.0102	.1820	.1012
11	.0061	.1436	.0780
12	.0036	.1114	.0596
13	.0021	.0866	.0454
14	.0012	.0665	.0344
15	.0007	.0507	.0260
16	.0004	.0385	.0197
17	.0002	.0292	.0148
18	.0001	.0221	.0111
19	.0001	.0166	.0084
20	.0000	.0125	.0063

TABLE III.

*Number of plants with 20 seeds or less.*

No. of seeds	A	B	C	D
5	—	1	—	—
6	—	—	1	—
7	—	1	3	2
8	3	2	—	1
9	2	1	1	1
10	1	3	3	1
11	5	2	4	1
12	3	1	2	1
13	2	—	3	—
14	2	3	—	4
15	2	3	1	—
16	8	1	4	—
17	3	2	6	7
18	3	4	4	1
19	2	—	2	3
20	5	2	2	1
Total	41	26	36	23

From the work that follows, I think the main explanation of the divergence must be in some way genetic. But there are certainly points

<sup>1</sup> The point might however become of considerable importance in classifying litters in which the number never exceeds a few units.

which suggest that part at least of the explanation may lie in differential death-rate.

Table IV compares the proportion per 1000 of seeds sown in each generation that survived to give plants included in the record with the value of  $P$  from Table I. If the plant was not included in the record it may be either that it did not give any viable plant at all—which would account for the majority of omissions—or it gave a plant with no seeds, or a plant with so few seeds that, while it was duly entered in the original sheets, it was omitted from the tables now discussed; in every generation there was a scattering of such plants. If, then, for brevity we speak of the proportions in the fourth column of Table IV as the “proportions of survivals” it must be remembered that this phrase is not strictly correct.

Comparing the last two columns of the table, it will be seen that in generations  $G$  and  $H$  where the values of  $P$  are the lowest observed, the proportions of “survivals” are also the lowest observed; and in generation  $M$  where the value of  $P$  is the highest observed, the proportion of “survivals” is also the highest observed. With the single exception of generation  $L$  the order of the values of  $P$  is the order of the “proportions of survivals.” While it does not, of course, amount to proof

TABLE IV.

*Comparison of the values of  $P$  from Table I with the proportions of seeds surviving.*

Crop	Seeds sown	Plants in record	Plants recorded per 1000 sown	$P$
$G$	460	371	807	·0004
$H$	390	334	856	·018
$J$	330	311	942	·17
$K$	330	307	930	·11
$L$	320	295	922	·37
$M$	310	296	955	·66

TABLE V.

*Comparing the numbers of plants of each type in the aggregate of the six crops with the numbers expected from the seeds sown and with the numbers to be expected from the total that would give the observed number of  $D$  plants.*

Type of plant	No. observed	Expectation from 2140 seeds sown	Expectation from number required to give $D$ 's observed	Proportion per 1000 of 2 on 4
1	2	3	4	5
$A$	775	951	1068	726
$B$	423	475·5	534	792
$C$	448	475·5	534	839
$D$	267	238	267	1000
Total	1913	2140	2403	—

this consilience suggests that the proportion of survivals is one factor at work influencing the value of  $P$ . Where the proportion of survivals is low, it suggests, the death-rate—or the failures to form plants with more than a bare minimum of seeds—falls differentially on the different types of seed, most heavily on the  $A$ 's (as a rule) and least heavily on the  $D$ 's, thus upsetting the simple expected Mendelian proportions.

If we regard, as above, all plants that fail to yield sufficient seed for them to be included in the record as "dead," the death-rates shown in Table IV range from 4.5 to no less than 19.3 per cent. These are "death-rates" falling on the plants from the seed stage onwards, though some seeds may have been dead when sown.

But there is a difficulty: in Table V, columns 2 and 3, the numbers of the four types of plant in the aggregate of the six generations are contrasted with the numbers to be expected from the numbers of seeds sown. It will be seen that the number of  $D$  plants recorded, viz. 267, is actually *greater than the number to be expected from the seeds sown*, viz. 238. This suggests that during the life-stage in the pod, if the suggested line of explanation is correct, differential death-rates must have been operating in precisely the same way as in the later life-stage; so that amongst the ripe seeds there was already a relative excess of  $D$ 's and deficiency of  $A$ 's. If we assume that all the  $D$  type fertilised ovules survived, we can estimate minimum death-rates for the other types. The work is carried out in the last two columns of Table V. The number of plants required to give 267 of type  $D$  is  $9 \times 267$  or 2403, and this total should be distributed over the four types as shown in col. 4. Col. 4 shows an expectation, for example, of 1068  $A$  plants, but there were only 775 observed; there was therefore a "survival rate" of only 726 per thousand, or a death-rate of 27.4 per cent. on the given test. Possibly we are in some degree exaggerating deaths, for the excess of  $D$  plants observed may have been partly, though only partly, a mere fluctuation of sampling. But subject to this caution the death-rates implied by col. 5 are the lowest possible values of the death-rates on this hypothesis: if all the  $D$  type fertilised ovules did not survive, and it is highly unlikely that they did, the survival-rates of col. 5 must be correspondingly lowered or the death-rates increased. This line of explanation would imply then that there must have been a death-rate, or its equivalent, of at least 28 per cent. on the  $A$  ovules and seeds—taking the plants through the whole course of their life up to the stage of the opening of flowers—and of at least 16 to 20 per cent. on the  $B$  and  $C$  ovules and seeds, against the minimum of no death-rate at all on the  $D$  ovules and seeds. The figures are so large as to be scarcely credible, though I doubt if they can

be called impossible. For the seeds that were sown in 1913 and gave the plants of Crop *G* there was a death-rate of nearly 20 per cent. on the four types together, from the sowing stage onwards.

I was at first inclined, notwithstanding the magnitude of the suggested death-rates, to regard this possible explanation as adequate. But the data and discussion of the following sections seemed practically to rule it out, at least as more than a subsidiary cause; the remarks of Mr Bateson and Miss Killby in the *Second Report of the Evolution Committee of the Royal Society* (cf. below, p. 275) suggest some observational evidence for differential death-rates. In the first place, if death-rates are so heavily differential as between the several types of fertilised ovule which all develop to YR seeds, they must almost inevitably be even more largely differential as between fertilised ovules which develop respectively to YR, YW, GR and GW seeds. This would completely upset the proportions of these seeds, and the data would diverge much more largely from Mendelian expectation than is in fact the case. In the second place, the evidence that follows seems to indicate that we cannot in fact postulate the simple Mendelian mechanism in its ideal form, and the explanation breaks down at its foundation. Notwithstanding Table IV, I am inclined to think the explanation must be in the main genetic.

The only other data I know giving the numbers of the four types of plant developed from YR seeds are Mendel's original figures:

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	Total
Observed	138	60	65	38	301
Expectation	133.8	66.9	66.9	33.4	301

Here both *A*'s and *D*'s are slightly in excess, and both *B*'s and *C*'s slightly deficient. But the differences are not significant, for *P* is 0.68. The number of seeds sown was 315, and the number of plants with seeds produced was 301 (11 seeds yielding no plants, and three plants with no seeds) so that the proportion of "survivals" from the seeds to the record was 956 per thousand. This proportion of survivals is almost identical with that for Crop *M*, which also gave no significant divergence from Mendelian expectation, and also gave slight excesses of *A*'s and *D*'s as in Mendel's data. The results are thus quite closely in accordance with one of our crops, though not with the general average.

#### *Summary.*

The four possible types of plant, dihybrids, hybrids for shape, hybrids for colour, and pure dominants, do not occur in the proportions required by the simple Mendelian theory.

Though these proportions fluctuate from crop to crop, the fluctuations lie within the limits of sampling from the average.

The average proportions are 404 : 221 : 234 : 140, against the Mendelian expectation of 4 : 2 : 2 : 1 or 445 : 222 : 222 : 111 per thousand. There is a deficiency of dihybrids and an excess of pure dominants.

Some examination of the data suggests that the divergence from Mendelian expectation may be due to differential death-rates. Tentatively, that explanation is rejected, at least as the main explanation, though it may be a subsidiary cause.

### III. THE PROPORTIONS OF SEEDS ON EACH TYPE OF PLANT IN EACH GENERATION AS A WHOLE.

Tables VI (A), VI (B), VI (C) show the numbers of seeds of each type counted, in each crop, on *A* plants, *B* plants and *C* plants respectively. Below the observed numbers of seeds are placed the "expected" numbers on the simple Mendelian ratios 9 : 3 : 3 : 1 and 3 : 1 respectively, and in the last column of each table is given the value of *P*, the probability that a fit as bad as or worse than that observed might be obtained on random sampling from a Mendelian population with the constitution postulated. For Table VI (A) the value of *P* is given by the  $\chi^2$  method. In the case of Tables VI (B) and VI (C) the standard deviation of random sampling was calculated throughout from the theoretical proportions, i.e. was taken as

$$s = \sqrt{\frac{1}{4} \times \frac{3}{4} N} = 0.43301N$$

and the value of *P* was found from the ratio of the observed deviation to *s* and tables of the normal function. With the large numbers that we have here to deal with the assumption of normality probably gives results of quite sufficient accuracy for the present purpose.

If the reader will turn first to Tables VI (B) and VI (C) he will notice that for no single crop is *P* smaller than 0.05 (Crop *J* of the *C*'s); i.e. even in this case one might expect a divergence from expectation as great as or greater than that observed once in some twenty trials on random sampling. For the *B*'s, though no one value of *P* is smaller than 0.14, no value is greater than 0.49 and the average value of *P* is about 0.29 only. This might suggest some source of discrepancies other than mere fluctuations of sampling; but when the six crops are aggregated the agreement with expectation is excellent, the value of *P* being 0.47—one might expect a worse agreement, on random sampling, at almost every other trial. Any definite discrepancies there may be in the different crops are, therefore, small and casual—sometimes in one direction and sometimes in the other.

In the case of the *C*'s, though the discrepancies in the individual crops are small and the average value of *P* as high as 0.53, the discrepancy in the aggregate of the six crops is marked and *P* is slightly under 0.1. Every crop but the last in fact shows an excess of greens, so that on the total there is an excess of over 140 green seeds. Considered in proportion to the total this deficiency is however not large, being just under 1.5 per cent. In view of the consistence of the different crops it may indicate some small real effect, but is still within the bounds of fluctuations of sampling. Considering these two tables as a whole there is little to explain; they give a series of quite fair examples of the fundamental 3 : 1 ratio.

But now, turning to Table VI (A) we see a very different story. Two crops, *J* and *K*, with over 17,000 seeds in each, give quite excellent agreement with the 9 : 3 : 3 : 1 ratio, much better agreements than are given by the first two crops. But *L* gives a very bad agreement, and *M* a divergence so great that the probability of getting as bad a fit on random sampling can only be expressed as a high negative power of 10—it lies between  $10^{-13}$  and  $10^{-14}$ . The most marked discrepancies are an excess of YW's and a deficiency of GW's, to the amount of about 200 in each case, but there is also a deficiency of GR's so that greens generally are deficient. If the six crops are pooled, as in the bottom section of the table, the deficiency of GW's is emphasised, as this is a feature common to every crop but *K*, but the excess of YW's is decreased as the four crops *G* to *K* show a deficiency. Next to the defect of GW's the most marked divergence in the general total is the excess of YR's, again a feature common to every crop but *K*. As shown by the value of *P*, on random sampling we would only expect a misfit as bad as that given by the total of the six crops once in some 25,000 trials; the divergence is almost certainly significant—but of what? The sum of the  $\chi^2$ 's is 91.51 which with  $n' = 19$  gives a vanishingly small value of *P* and emphasises the divergence.

The crops in this case diverge from one another as well as from Mendelian expectation. Treating Table VI (A) as a contingency table (cf. the treatment of Table I),  $\chi^2$  is 71.28,  $n' = 16$ , and *P* is again almost vanishingly small.

In this case we have earlier data from other observers which may be illuminating. In the first place there are Mendel's own figures<sup>1</sup>. He obtained excellent agreement, though with a sample much smaller than those with which we have to do in Table VI. *P* works out at 0.91, using the precise values for the expectations and not the rounded figures given

<sup>1</sup> Bateson, *Mendel's Principles*, p. 60.

TABLE VI (A).

*Total numbers of seeds of each type on the A plants of each crop; expected numbers on 9:3:3:1 ratio; and probability P of obtaining in random sampling a divergence as bad as or worse than that shown.*

Crop	Seeds					P and $\chi^2$
	YR	YW	GR	GW	Total	
G Obs.	3425	1059	1177	356	6017	P .049
The.	3385	1128	1128	376	—	$\chi^2$ 7.88
H Obs.	5383	1785	1820	556	9544	P .35
The.	5368.5	1789.5	1789.5	596.5	—	$\chi^2$ 3.33
J Obs.	9872	3251	3323	1074	17520	P .75
The.	9855	3285	3285	1095	—	$\chi^2$ 1.22
K Obs.	9984	3299	3345	1188	17766	P .78
The.	9993	3331	3331	1110	—	$\chi^2$ 1.09
L Obs.	4752	1575	1469	464	8260	P .0068
The.	4646	1549	1549	516	—	$\chi^2$ 12.23
M Obs.	7993	2846	2564	677	14080	P $3.5 \times 10^{-14}$
The.	7920	2640	2640	880	—	$\chi^2$ 65.76
G Obs.	41409	13815	13698	4265	73187	P .00004
to M The.	41167	13723	13723	4574	—	$\chi^2$ 22.95
G Distribution to M per 10000	5658	1888	1872	583	10000	—
	5625	1875	1875	625	—	—

TABLE VI (B).

*Total numbers of seeds of each type on the B plants; expected numbers on the 3:1 ratio; and probability P of obtaining in random sampling a divergence as great as or greater than that shown.*

Crop	Seeds			P
	YR	YW	Total	
G Obs.	3492	1107	4599	.14
The.	3449	1150	—	—
H Obs.	3857	1229	5086	.17
The.	3814.5	1271.5	—	—
J Obs.	5192	1691	6883	.40
The.	5162	1721	—	—
K Obs.	6032	2078	8110	.19
The.	6082.5	2027.5	—	—
L Obs.	3689	1268	4957	.34
The.	3718	1239	—	—
M Obs.	4485	1464	5949	.49
The.	4462	1487	—	—
G Obs.	26747	8837	35584	.47
to M The.	26688	8896	—	—
G Distribution to M per 10000	7517	2483	10000	—
	7500	2500	—	—

TABLE VI (c).

*Total numbers of seeds of each type on the C plants; expected numbers on the 3 : 1 ratio; and probability P of obtaining in random sampling a divergence as great as or greater than that shown.*

		Seeds			P
Crop		YR	GR	Total	
G	Obs.	2744	939	3683	·50
	The.	2762	921	—	—
H	Obs.	3538	1204	4742	·54
	The.	3556·5	1185·5	—	—
J	Obs.	7550	2630	10180	·05
	The.	7635	2545	—	—
K	Obs.	6544	2189	8733	·88
	The.	6550	2183	—	—
L	Obs.	2993	1027	4020	·42
	The.	3015	1005	—	—
M	Obs.	5004	1657	6661	·82
	The.	4996	1665	—	—
G to M	Obs.	28373	9646	38019	·095
	The.	28514·5	9504·5	—	—
G to M	Distribution	7463	2537	10000	—
	per 10000	7500	2500	—	—

below. But this is in no way inconsistent with our own results, for divergences of the same proportionate amount as those found in the 70,000 observations of the aggregate of the six crops would be totally insignificant on a sample of this size. As a matter of fact—I do not stress it of course—the GW's *are* in defect in Mendel's data, the deficiency

	YR	YW	GR	GW	Total
Observed	315	101	108	32	556
Expected	313	104	104	35	—

reckoned on the precise expectation of 34·75 being 8·0 per cent. In the aggregate of our six crops we have 4265 GW's against an expectation of 4574, and that is a deficiency of only 6·8 per cent. Again, YR's are actually in excess in Mendel's data, by 0·72 per cent.; in the aggregate of the six crops they are only in excess by 0·59 per cent. In both instances it is the very large number of observations in the Darbishire record that makes the small relative deviations significant and important. If the actual distribution of Mendel's figures be multiplied up in the ratio of 73,187 to 556, the resulting distribution would give a value of *P* of the order  $10^{-15}$ , a value nearly as low as that of our distribution in Crop *M*. Conversely, if Crop *M* had had only 556 observations instead of 14,080, the value of  $\chi^2$  would have been reduced from 65·76 to 2·60 and *P* would have been 0·46—the observations would have appeared to be quite a



good fit to Mendelian expectation. The importance of the number of observations must be borne in mind; the deviations are *relatively* small.

A much larger series of observations is available in the *Second Report of the Evolution Committee of the Royal Society*, p. 77.

	YR	YW	GR	GW	Total
Observation	4926	1656	1621	478	8681
Expectation <sup>1</sup>	4883.0	1627.7	1627.7	542.6	—

The distribution here rather closely resembles our total for the six crops, the signs of the deviations being the same. There is an excess of 0.98 per cent. of YR's and a deficiency of 12 per cent. of GW's. Dr Bateson and Miss Killby suggest an explanation of the deficiency of GW's which is the most conspicuous feature: "The net deficiency of green wrinkled seeds is probably due to a slightly greater tendency to tinge, or to burst, owing to which a larger proportion have been consigned to the 'dubious' class, or have been rejected as dead." There are two explanations here, (a) difficulty of classification, leading to the seeds being queried instead of being allocated to their class, (b) a differential death-rate falling more heavily on the GW seeds.

The number of seeds classed as doubtful in Dr Bateson and Miss Killby's record is large, viz. 348, so that if it is legitimate appropriately to distribute them the divergences observed can be greatly reduced or even eliminated. But examination of the data, given in full in the *Report*, has not convinced me that the greater part of the discordance can be explained on these lines. Selecting from the data series with relatively few doubtfuls still leaves a deficiency of GW's. I tried more than one mode of selection, e.g. rejecting any series which had a total of "dubious" equal to or greater than one-eighth of the total of heterozygotes. The test left me with 5809 seeds or 67 per cent. of the total, and only 94 doubtfuls or 27 per cent. of the original number, so that if the suggested explanation were correct a considerable improvement in fit with expectation should have been effected. The figures obtained were:

	YR	YW	GR	GW	Total
Observation	3237	1121	1122	329	5809
Expectation	3267.6	1089.2	1089.2	363.1	—

The deficiency of GW's is now 9.4 per cent. against 12 per cent. The values of  $\chi^2$  and  $P$  for the two tables are:

	$\chi^2$	$P$
8681 observations	8.59	0.036
5809       ,,	5.41	0.147

<sup>1</sup> The values given in the *Report* are in error by a few units.

But the increase in the value of  $P$  is mainly due to the reduction in the number of observations. If 5.41 be multiplied in the ratio of 8681 to 5809 it is raised to 8.08, which would give  $P = 0.045$ —a comparatively small improvement, partly because the three classes other than GW's do not agree so well with expectation as they did in the total.

If we further deduct from the above numbers the data due to a group with 58 dubious (from the cross, Exp. ♀ × Serp. ♂ under the number 32 in the tables of the *Report*) we obtain the following distribution:

	YR	YW	GR	GW	Total
Observation	1776	594	615	181	3166
Expectation	1780.9	593.6	593.6	197.9	—

The deficiency of GW's is now 8.5 per cent.;  $\chi^2$  is 2.22 and  $P$  is 0.53 as the observations stand, but if the number of observations were multiplied up to the original total of 8681  $\chi^2$  would be 6.09 and  $P$  0.11. We have thus steadily improved the fit by excluding dubious seeds, but not, as it seems to me, to anything like the extent we should have done if they were the main source of the original discrepancy. In this last series there were only 34 dubious seeds.

We may look at the matter another way, thus. The deficiency of GW's in the original data was 64.6, and the number of dubious seeds 348; to fill the gap in GW's 18.6 per cent. of the dubious seeds should accordingly be GW's. In the group that yielded 5809 sorted seeds there were only 94 dubious, and 18.6 per cent. of 94 is 17.5, but the deficiency of GW's is 34. In the group yielding 3166 sorted seeds there were 36 dubious, and 18.6 per cent. of 36 is 6.7, but the deficiency of GW's is 17. To fill the gaps in GW's in the successive series with fewer and fewer unsorted seeds we should have to assume that larger and larger proportions of these unsorted seeds were GW's. This does not seem reasonable.

Further, in our own data the exclusion of dubious seeds cannot be put forward as an explanation of discrepancies, as relatively few seeds had to be written down as dubious. If a seed was dubious in colour, as judged by external appearance, a small segment was removed with a sharp knife and the cotyledon colour was seldom doubtful. A few cases of shape might be doubtful owing to pitting, but not many. In the whole of the *A* plants of Crop *M*, I can only find 31 dubious seeds; there is a deficiency of 203 GW seeds. It may be said that seeds which *ought* to have been written down as dubious may have been mis-sorted; they cannot at least have been mis-sorted as YR's, or we should have found some of the YR seeds sown yielding plants with only green-wrinkled seeds. I have every reason to believe that the sorting was done carefully with full recognition of the difficulties.

There remains the theory of the *Report* that a differential death-rate is the source of disturbance. I gather from the wording that there is observational evidence to support this view. It is in accordance with the explanation tentatively put forward in the last section to explain the discrepancies in numbers of different types of plant, but I felt unable to retain that hypothesis for the reasons there stated.

To turn to another writer, Mr Orlando E. White<sup>1</sup>, in a paper entitled "Inheritance studies in *Pisum*," remarks: "The expression of factor *G* (green cotyledon pigment) fades on prolonged exposure to sunlight and wet weather, and seeds of wrinkled seeded varieties fade more quickly than those of round seeded forms. Lack of sufficient sunlight, immaturity due to prolonged growth of the vine, and other environmental factors affect the action of factor *I* (the factor which causes green cotyledon colour to fade)." This would seem to suggest the possibility of some mis-sorting resulting in a transfer of *GW*'s to the heading *YW*, and in a lesser degree of *GR*'s to *YR*, which would account for the facts observed in Crop *M*, but knowing the care taken in classification the adequacy of the explanation seems to me doubtful, and if some seeds classed as *YR*'s had been really *GR*'s we should have found this in the sowing.

Dr Bateson and Miss Killby, in the *Report of the Evolution Committee* cited above, only suggest the existence of disturbing causes, such as differential death-rates or failures to sort correctly, and not the inadequacy of the factor-mechanism postulated. But I feel compelled, by the present work, to postulate that inadequacy and start therefore from a different standpoint. I do not wish, however, to imply that such disturbing causes are not operative. I regard them only as subsidiary. Table VII, for the dihybrid plants of Crop *M*, shows that all kinds of disturbing elements may be at work. It will be seen that as the yield of the plant is increased from less than 50 seeds to 200 or more, the proportion of *GW*'s rises from an average of 448 per 10,000 to 494 per 10,000; while contrariwise the proportion of *YR*'s falls from 5766 per 10,000—an excess over Mendelian expectation—to 5264, a heavy deficiency.

### Summary.

The proportions of the seeds on dihybrids given by the total of the six crops differ significantly from Mendelian expectation.

The principal divergences consist of a deficiency of *GW* seeds, such as was noted in the *Second Report of the Evolution Committee of the Royal Society*, mainly compensated by an excess of *YR*'s.

<sup>1</sup> *Journal of Agricultural Research*, Vol. XI. 1917, p. 167.

The deficiency of GW's and excess of YR's is noted in every crop but one.

The crops differ, however, significantly from each other in the proportions given.

Differential death-rates again seem inadequate as an explanation of the facts, though it is not denied that such disturbing causes are in operation. In one crop tested, the proportions on dihybrid plants vary with the number of seeds on the plant.

For the monohybrids results are close to expectation, but there seems to be some tendency in monohybrids for colour to a small deficiency of yellows.

TABLE VII.

*Actual numbers of seeds on A plants with different total numbers of seeds, and distributions per 10,000 seeds, in Crop M.*

No. of seeds on plant	Observed numbers				
	YR	YW	GR	GW	Total
Under 50 ...	489	168	153	38	848
50 but less than 100	1703	609	522	136	2970
100 but less than 200	5034	1743	1597	431	8805
200 and over ...	767	326	292	72	1457
Per 10,000					
Under 50 ...	5766	1981	1804	448	10000
50 but less than 100	5734	2050	1758	458	10000
100 but less than 200	5717	1980	1814	489	10000
200 and over ...	5264	2238	2004	494	10000
Expectation ...	5625	1875	1875	625	10000

#### IV. THE PROPORTIONS OF SEEDS ON INDIVIDUAL PLANTS OF EACH TYPE.

The present section is devoted to the study of the fluctuations in the proportions of seeds, of each sort, from plant to plant. We "expect," for example, 56.25 per cent. of YR seeds on an A plant, though we know we never get—except rarely by a mere chance—precisely this percentage. But how do the percentages vary? Do we find only the amount of variation to be expected on the theory of simple sampling? or more? If the frequency distributions of percentages are plotted, do they show only the characteristics to be expected on the theory of simple sampling or any suggestive divergences? These are the types of question that arise.

In previous work on this subject<sup>1</sup>, to which I shall have occasion to

<sup>1</sup> Yule, "Fluctuations of sampling in Mendelian ratios," *Proc. Camb. Phil. Soc.* Vol. xvii. 1914, p. 425. The suggestion of limiting the study of the question to the case of plants with a certain minimum of seeds was originally made by Mr Darbishire himself, on the ground that such plants might be diseased or exceptional. But the reasons are, I think,

refer again later, it was found desirable to confine the study of this kind to plants with a certain minimum of seeds. Plants with few seeds inevitably show a range of variation so wide that not only may the results be erratic, but any special characteristics of the distribution may be masked by casual fluctuations and irregularities. What is gained in numbers by including plants with few seeds is lost—if we are endeavouring to trace any definite sources of divergence—by the overwhelming weight of chance variations. As a rule I have therefore confined myself to the study of plants with 100 seeds or more. Even out of this population, as will be seen in the sequel, I have found it desirable further to select the plants with a minimum of 200 seeds, notwithstanding the consequent limitation of numbers, for special study.

Tables VIII (A), (B) and (C) give a summary view of the frequency distributions of percentages, for the six crops as a whole; and Tables IX (A), (B) and (C) show the numbers of plants from each crop that contributed to the preceding table, together with the numbers of seeds on them. Table VIII is read as follows, taking (A) as an illustration. From the row with the side-heading "Total" near the bottom it will be seen that there were 285 plants with 100 seeds or more (*A* plants, dihybrids) in the aggregate of the six crops. Of these 285 plants one bore no more than 45 per cent. of YR seeds, while one bore 70 per cent. Forty-two plants bore 58 per cent. of YR seeds, and this is a larger number than bore any other single percentage. The "expected" percentage is 56.25 or 56 to the nearest unit, and only 26 plants showed this percentage. Referring to the bottom of the table, the mean percentage calculated from the frequency distribution is 56.74, which differs from the expected value by 0.49 or nearly twice the standard error of the mean, given in the lowest section of the table. Below the mean  $\bar{M}$  is given the standard deviation  $s$ ; the standard error of the mean is  $s/\sqrt{N}$ , where  $N$  is the number of observations, and the standard error of the standard deviation has been taken at its value for a normal distribution, viz.  $s/\sqrt{2N}$ , which is given in the last line of the table. The other columns of this table, and the columns of Table VIII (B) and (C) are read similarly. The calculation of  $s_0$ , the standard deviation of sampling, is explained below.

Before turning to the consideration of Table VIII some minor points are of interest. It will be seen from Table IX that the different crops contribute to Table VIII in proportion neither to the total numbers of

wider than this, especially in the present case where we want to study the form of the frequency distribution.

I am not sure, however, that something may not have been lost by such exclusion if plants tend to be small simply because they are exceptional.

seeds harvested (Table VI) nor to the total number of plants harvested (Table I). Crop *G* is particularly deficient in plants with 100 seeds or more, but this, I understand, is an artificial result due to the fact that Mr Darbishire did not always count out all the seeds on a plant but stopped when he reached an arbitrary minimum; such a limitation can be understood when the average proportions were regarded as the main object of determination, but from the present standpoint was unfortunate. Later generations were completely counted. Comparing the numbers of plants with 100 seeds or more with the totals of Table I, the figures run as follows:

*A*'s, 285 out of 775, or 37 per cent.

*B*'s, 122 out of 423, or 29 per cent.

*C*'s, 137 out of 448, or 31 per cent.

TABLE VIII (A).

*Number of A plants with 100 seeds or more, in the total of the six crops, with each percentage of YR, YW, GR and GW seeds; together with the mean percentage M; the s.d., s; and the s.d. of sampling s<sub>0</sub> based on the mean percentage observed.*

Per-centage	No. of plants with said percentage of YR seeds	Per-centage	No. of plants with said percentage of YW seeds	No. of plants with said percentage of GR seeds	Per-centage	No. of plants with said percentage of GW seeds
45	1	9	1	1	1	3
46	—	10	—	—	2	5
47	1	11	2	1	3	23
48	4	12	5	3	4	47
49	6	13	3	11	5	48
50	8	14	8	15	6	68
51	17	15	21	16	7	40
52	17	16	25	20	8	28
53	13	17	34	31	9	12
54	16	18	37	40	10	7
55	16	19	37	35	11	1
56	26	20	33	31	12	2
57	26	21	27	30	13	1
58	42	22	15	22	—	—
59	30	23	14	16	—	—
60	14	24	8	5	—	—
61	16	25	4	5	—	—
62	9	26	2	2	—	—
63	9	27	2	—	—	—
64	2	28	4	1	—	—
65	2	29	2	—	—	—
66	6	30	1	—	—	—
67	3	—	—	—	—	—
68	—	—	—	—	—	—
69	—	—	—	—	—	—
70	1	—	—	—	—	—
Total	285	—	285	285	—	285
<i>M</i>	56.74	—	18.88	18.62	—	5.78
<i>s</i>	4.221	—	3.364	3.095	—	1.967
<i>s</i> <sub>0</sub>	4.040	—	3.192	3.175	—	1.904
S.e. of <i>M</i>	.250	—	.199	.183	—	.117
S.e. of <i>s</i>	.177	—	.141	.130	—	.082

The *A*'s therefore seem to have an appreciably possibly significant, larger proportion of vigorous plants. The *D* plants, bearing only YR seeds, do not come under consideration here, but a search of the lists shows only 21 plants with 100 or more seeds out of 267, or no more than 8 per cent.

In view of the varied weighting of the different crops in the total a sensible alteration might be expected in the proportions of seeds of each type, but the actual changes are very small. The following are the proportions per 10,000 of seeds on the *A* plants:

	YR	YW	GR	GW	Total
All plants ... ..	5658	1888	1872	583	10000
With 100 seeds or more	5658	1893	1865	584	10000

TABLE VIII (B) AND (C).

*Numbers of B and C plants with 100 seeds or more, in the total of the six crops, with each percentage of YR seeds; together with the mean percentage  $\bar{M}$ , the s.d.,  $s$ ; and the s.d. of sampling  $s_0$  based on the mean percentage observed.*

Percentage	(B) No. of plants with said percentage of YR seeds (R : W)	(C) No. of plants with said percentage of YR seeds (Y : G)
61	—	1
62	1	—
63	—	—
64	—	1
65	1	—
66	1	2
67	2	—
68	1	2
69	5	5
70	5	2
71	3	12
72	10	10
73	11	14
74	11	10
75	12	13
76	20	17
77	13	16
78	7	18
79	6	4
80	5	3
81	4	2
82	3	2
83	—	1
84	1	1
85	—	1
<hr/>		
Total	122	137
<hr/>		
$\bar{M}$	74.88	74.81
$s$	3.789	3.770
$s_0$	3.562	3.572
<hr/>		
S.e. of $\bar{M}$	.343	.322
S.e. of $s$	.243	.228

TABLE IX (A).

*Plants with 100 seeds or more; numbers of A plants and their seeds in each crop.*

Crop	Plants	Seeds				Total
		YR	YW	GR	GW	
<i>G</i>	8	563	167	188	51	969
<i>H</i>	22	1817	611	591	188	3207
<i>J</i>	76	7869	2579	2608	854	13910
<i>K</i>	82	8611	2841	2895	964	15311
<i>L</i>	27	2018	657	623	196	3494
<i>M</i>	70	5801	2069	1889	503	10262
Total	285	26679	8924	8794	2756	47153
Expectation	—	26524	8841	8841	2947	—
Distribution per 10,000 observed	—	5658	1893	1863	584	10000

TABLE IX (B).

*Plants with 100 seeds or more; numbers of B plants and their seeds in each crop.*

Crop	Plants	Seeds		
		YR	YW	Total
<i>G</i>	3	280	85	365
<i>H</i>	15	1551	515	2066
<i>J</i>	27	3642	1189	4831
<i>K</i>	38	5039	1746	6785
<i>L</i>	14	1325	421	1746
<i>M</i>	25	2983	980	3963
Total	122	14820	4936	19756
Expectation	—	14817	4939	—
Dist. per 10,000 observed	—	7502	2498	10000

TABLE IX (c).

*Plants with 100 seeds or more; numbers of C plants and their seeds in each crop.*

Crop	Plants	Seeds		
		YR	GR	Total
<i>G</i>	2	179	51	230
<i>H</i>	12	1202	419	1621
<i>J</i>	41	5780	1988	7768
<i>K</i>	43	5473	1838	7311
<i>L</i>	9	888	311	1199
<i>M</i>	30	3190	1047	4237
Total	137	16712	5654	22366
Expectation	—	16774.5	5591.5	—
Dist. per 10,000 observed	—	7472	2528	10000



The *B* and *C* plants compare as follows:

	<i>B</i> plants		
	YR	YW	Total
All plants ... ..	7517	2483	10000
With 100 seeds or more	7502	2498	10000

	<i>C</i> plants		
	YR	GR	Total
All plants ... ..	7463	2537	10000
With 100 seeds or more	7472	2528	10000

There is therefore very little difference, in the total, between the proportions of seeds on plants with 100 seeds or more and the proportions of seeds in the general aggregate.

In the average proportions given by Tables IX each plant is weighted in proportion to the number of seeds it bears; in the averages of Tables VIII the percentage of each sort of seed borne by a plant is counted only once. Hence comparison of the two averages will show whether larger or smaller percentages are, on the whole, associated with the more prolific plants; the comparison given above suggests very little correlation of the kind notwithstanding the results shown by Table VII for Crop *M*. The averages for *A* plants compare as follows:

	YR	YW	GR	GW	Total
Average weighted	5658	1893	1865	584	10000
„ unweighted	5674	1888	1862	578	10000

This comparison shows that on the whole the more prolific plants bear fewer YR's and more GW's, a result in the same direction as that given by Table VII, but the difference is small and is less than the standard error in each case. For *B* plants the averages are:

	YR	YW	Total
Average weighted	7502	2498	10000
„ unweighted	7488	2512	10000

In the case of the *B*'s the more prolific plants bear relatively more YR's—i.e. fewer wrinkled seeds. The averages for the *C*'s run:

	YR	GR	Total
Average weighted	7472	2528	10000
„ unweighted	7481	2519	10000

Here again the more prolific plants, as in the dihybrids, bear relatively fewer YR's, i.e. relatively more green seeds, than the less prolific plants. The differences are not, however, large.

We now turn to a comparison of the actual fluctuations observed with the fluctuations of sampling. The first comparison was effected simply in terms of the standard deviation. If, say, we draw  $n$  counters at a time

out of a very large number of red and white counters mixed in the proportion  $p$  to  $q$ , drawing purely at random, the standard deviation of the proportions of red counters observed in the samples will approach, within the limits of fluctuations of sampling, the value  $\sqrt{pq/n}$ , where  $p$  and  $q$  can be expressed as percentages if it is desired to obtain the answer in terms of percentages. If we had a large number of plants and took the same number of seeds  $n$  from each, and if the Mendelian proportions were subject to no cause of variation except fluctuations of sampling, we should expect the standard deviation of the percentages of any given type of seed to be given by the above expression.

But in practice we have not got the same number of seeds on each plant;  $n$  varies from plant to plant. Clearly in this case we have to substitute for  $n$  some sort of average of the number of seeds per plant. The average to be used is the harmonic mean (the reciprocal of the arithmetic mean of the reciprocals); in other words we substitute the arithmetic mean of  $1/n$  for  $1/n$  in the formula<sup>1</sup>. This is the expression symbolised by  $s_0$  in Tables VIII, i.e.

$$s_0^2 = pq/H,$$

where  $H$  is the harmonic mean of the numbers of seeds on the plants included in the table. The values of  $1/H$  and of  $H$  are as follows:

*A* plants:  $1/H$ , 0.0066516;  $H$ , 150.3.

*B* plants:  $1/H$ , 0.0067467;  $H$ , 148.2.

*C* plants:  $1/H$ , 0.0067701;  $H$ , 147.7.

$H$  was determined by writing down the reciprocal of  $n$  for each plant, to two significant figures, from Barlow's Tables, and averaging. For the  $p$  and  $q$  of the formula it makes very little difference whether we use the expected Mendelian percentages or the average percentages actually observed; the latter were used.

Looking through the values of  $s$  and  $s_0$  in Tables VIII (A), (B) and (C) we see that in every case but one the value of  $s$  exceeds that of  $s_0$ ; the exceptional distribution is that for GR seeds on *A* plants. In the other cases the excess is not great, only in two distributions out of five slightly exceeding the standard error of the standard deviation. Taking the figures as a whole they suggest a small influence of some definite cause or causes tending slightly to increase the range of fluctuations of sampling. If two causes of fluctuation, acting independently, are superposed the squares of their standard deviations add, so that the standard deviation of the percentages due to definite causes will be given by  $\sqrt{s^2 - s_0^2}$ . The values of this expression for the YR's, YW's and GW's of Table VIII (A)

<sup>1</sup> Cf. Yule, *Introduction to the Theory of Statistics*, Chap. XIII. Section 11.

are 1.223, 1.062, 0.494; for the proportions of YR's on *B* and *C* plants respectively 1.292 and 1.196. If we are justified in assuming that the exceptional case of the GR's on *A* plants is due to the chances or sampling—and the negative difference is only some three-fifths of the standard error of the standard deviation—the figures suggest some sort of disturbing cause affecting the percentages to the extent of a standard deviation of about 1.2 units in the case of YR's on *A* plants, of probably less than a unit in the case of YW's and GR's on the same plants, and of about half a unit in the case of GW's. The percentages of YR's on *B* and *C* plants seem to be affected to about the same extent as the percentages of YR's on *A* plants.

To attempt to throw a little more light on the matter I wished to compare the actual distribution with that to be expected on the theory of sampling. As is well known, the frequencies of the actual numbers of red counters—to refer to our previous illustration—in a sample of  $n$  should be given by the terms of the binomial

$$(q + p)^n,$$

where  $p$  is the proportion of red counters in the "universe" from which drawings are made and  $q$  is  $1 - p$ . If  $n$  is large, as in our present case, calculations may be simplified by using Prof. Pearson's approximation to the binomial

$$y = y_0 e^{-\gamma x} \left(1 + \frac{x}{a}\right)^{\gamma a}.$$

But matters are again complicated by the fact that  $n$  is not constant. It would have been an exceedingly laborious matter to break up the population of plants into a series of small sections each with nearly the same number of seeds—say 100 to 119, 120 to 139, and so on—and to calculate a separate curve for each, adding them together to get a curve for the aggregate, and I adopted a simpler approximation, merely calculating the curve corresponding to  $H$  the harmonic mean value of  $n$ . This is recognised to be a rough process, but probably gives a sufficiently close approximation. The values assigned to  $p$  and  $q$  were the values on Mendelian expectation, so that the whole curve was calculated on an *a priori* basis.

The formulae for the curve constants in terms of the binomial constants are as follows:

Mode at  $c(np - q + 0.5)$ ,

$$\gamma = \frac{2}{c(q - p)},$$

$$a = \frac{2pq(n + 1)c}{q - p},$$

where  $c$  is the distance apart of the binomial ordinates; as we plot to a scale of 1 per cent.,  $c$  is  $100/H$ . As already stated  $H$  was substituted for  $n$ , and the *a priori* values were used for  $p$  and  $q$ , e.g. 9/16 and 7/16 in the case of YR's on  $A$  plants. The constants  $a$  and  $\gamma$  are thus determined, and the position of the mode, to which the equation for the curve is referred. For  $y_0$  it is sufficient to use the approximate formula

$$\log y_0 = \log N + \frac{1}{2} \log \gamma - \left( \log \sqrt{2\pi} + \frac{1}{12a\gamma} \log e + \frac{1}{2} \log a \right),$$

$N$  being the number of observations.

In the case of the distributions for  $B$  and  $C$  plants the values of  $H$  are so nearly identical that after the former had been calculated it was thought sufficient to use it for the latter as well. The constants determined are as follows:

	$y_0$	$\gamma$	$a$	Mode
A. YR	28.01	-24.05	-396.4	56.29
A. YW and GR	35.59	+4.810	+49.07	18.54
A. GW	57.29	+3.435	+13.48	5.96
B and C. YR	13.64 and 16.77	-5.928	-75.51	75.40

The observed distributions with the calculated curves superposed are shown in Figs. 1 to 6, pp. 259, 260. Testing the "goodness of fit" by the  $\chi^2$  method<sup>1</sup> we find the following results for the grouping stated:

	$n'$	$\chi^2$	$P$
A. YR, 49 and under, 64 and over, grouped	16	28.31	0.020
A. YW, 12 and under, 26 and over, grouped	15	11.49	0.65
A. GR, 12 and under, 26 and over, grouped	15	9.61	0.79
A. GW, 2 and under, 11 and over, grouped	10	21.25	0.012
B. YR, 68 and under, 81 and over, grouped	14	10.07	0.69
C. YR, 68 and under, 81 and over, grouped	14	17.30	0.19

For the seeds on dihybrid plants the YW and GR distributions, Figs. 2 and 3, p. 259, give a very fair agreement with the calculated curves. The charts show no very conspicuous or continuous divergences between observation and calculation; such as they are, attention may be directed to a small continuous deficiency of YW's between 22 and 26 per cent., compensated by a small but rather conspicuous excess at 27 per cent. and over, and to the irregularity on the left of the GR distribution with the excesses at 13 and 14 per cent. rather suggestive of a hump in the curve.

The distributions of the GW and YR percentages on the dihybrids are very different, both giving a low value of  $P$  and a very poor fit. Taking the GW's (Fig. 4) first, the reason for the low value of  $P$  is seen to lie in a general shift of the distribution to the low end of the scale, a shift

<sup>1</sup> Calculated ordinates were corrected to areas by adding one-twenty-fourth of the second difference for the three ordinates of which the given one was the central.

that might have been expected in view of the low value of the mean, 5.78 per cent. against the Mendelian value 6.25. The distribution also shows rather a marked hump on the left at 4 per cent., a little suggestive of the corresponding hump on the GR distribution.

Turning to the YR's (Fig. 1) we have quite a different story. The mean in this case is high, 56.74 against the expected 56.25, but the distribution as a whole cannot be said to be shifted to the right. The maximum frequency is given not by 56 or 57 per cent., as we would expect, but, very conspicuously, by 58 per cent. The whole distribution is also very erratic. Beginning at the lower end of the curve, we find that it passes under the observation-polygon at 48 per cent. and remains under up to 52 per cent., the observations showing a conspicuous peak at 51 to 52. From 53 to 57 per cent. there is a continuous deficiency of observations as compared with expectation amounting to no less than 29 on an expected total of 126. Then comes the marked peak at 58 per cent., followed by another excessive number of observations at 59 per cent., the combined excesses amounting to 24 observations on an expected total of 48—an excess of 50 per cent. From 60 to 65 per cent. the curve runs fairly through the observations, but from 66 per cent., inclusive, upwards there are ten observations against an expectation of little more than three. It looks as if we had here to do with some quite definite divergence from the expected distribution, more than would be suggested merely by the excess of the mean. The whole distribution looks compound. The value of  $P$  calculated by only grouping the tails of the distribution, low though it is, does not fully reflect (as will often happen) the eye-judgment of the significance of the divergences shown by the chart, since the order of these divergences is not taken into account by the  $\chi^2$  method. We get a much more significant figure if the distribution is grouped as above by the successive runs of excess and defect. The figures are as follows:

Percentage of YR's	Frequency		Difference	Diff. <sup>2</sup> /The.
	Actual	Theoretical		
Up to 47	2	4.59	- 2.59	1.46
48-52	52	46.04	+ 5.96	.77
53-57	97	125.96	- 28.96	6.66
58-59	72	47.90	+ 24.10	12.13
60-65	52	57.32	- 5.32	.50
66 upwards	10	3.19	+ 6.81	14.54
			Total	36.06

The value of  $\chi^2$  is 36.06 and  $n'$  is only 6, which gives for  $P$  roundly .000006; or a worse fit, owing merely to chances of sampling, would be expected only some six times in 1,000,000 trials. Practically speaking

this may be regarded as impossible; we are compelled to postulate that some definite and assignable cause has been at work.

An interesting result is given by breaking up the distribution into two parts, for plants with 100 to 199 seeds, and plants with 200 seeds or more. The figures are given in Table X. The distribution for the large plants is even more suggestive of a compound distribution than is the original aggregate (cf. Fig. 7*a*, p. 261). Of the 66 plants in this distribution, 56 belong to Crops *J* and *K*, the totals of seeds in which gave the best fits, and very good fits, to simple Mendelian expectation (cf. Table VI (A)). It seemed of interest therefore to subject these 56 plants to more detailed examination.

The frequency distributions of percentages of YR's, etc. are given in Table XI, in the same form as Table VIII (A). It will be seen that the standard deviation in each case exceeds the s.d. of sampling, though the excess for the GW's is very small. Further, the distribution of YR's remains just as clearly suggestive of two components, one centering round a value near 52 per cent., the other round a value about 57 to 58 per cent.; the distribution is illustrated in Fig. 7 *b*. On the other hand, the means of the several distributions, viz. 56.16, 18.86, 18.36, 6.32, are in fairly close accordance with the theoretical values 56.25, 18.75, 18.75, 6.25, and this closeness of agreement is confirmed by the totals of seeds in Table XII, which lie extraordinarily close to expectation,  $\chi^2$  working out at 0.72 only and *P* at 0.86, so that we might expect a worse agreement eight or nine times in ten trials.

Looking further into the distribution of percentages for the YR's, it would seem that, if there are two components, these may be divided roughly from each other at a point between 54 and 55 per cent. This would give 14 observations to the lower component and 42 to the upper, a ratio of exactly 3 : 1; but how can a 3 : 1 ratio arise in the present circumstances? The totals for the separate crops are:

	Under 55	55 and over	Total
<i>H</i>	1	3	4
<i>J</i>	9	17	26
<i>K</i>	5	25	30
<i>M</i>	4	2	6

but the figures are too small to lay any stress on the fluctuations. Nor is the 3 : 1 ratio the only puzzle. Taking the two distributions for the YR's of *J* and *K* alone as divided at 54.5, the means and s.d.'s work out at:

Lower distribution: Mean 51.38; s.d. 1.68

Upper distribution: „ 57.99; „ 1.75

This result presents us at once with a difficulty, for the s.d.'s observed are far smaller than the standard deviations of sampling. Ignoring the difference between the average numbers of seeds borne by the plants of the two groups, which Table XII shows to be very small, the s.d.'s of sampling are 3.17 and 3.13 respectively, as calculated from the observed means, against the observed figures 1.68 and 1.75. I have totally failed to conceive of any mechanism which could *so greatly* limit the s.d. of sampling, though the question has raised itself in my mind whether the distribution of factors amongst the ovules should be regarded as random. If not random but determinate, there would result a certain reduction in the s.d. of sampling for the zygotes but quite insufficient to explain such a result as the above.

If, putting aside this difficulty for the moment, we examine the distributions of seeds in the two groups, given in Table XII, we do not get much light on the matter. The distributions are not such as would be produced by slight positive coupling in the one case and negative in the

TABLE X.

*Analysis of the A plants with 100 seeds or more into plants with 100-199 seeds and plants with 200 seeds or more, as regards the distribution of YR's (Table VIII (A)).*

Percentage	Plants with		Total
	100 to 199 seeds	200 seeds or more	
45	1	—	1
46	—	—	—
47	—	1	1
48	3	1	4
49	5	1	6
50	6	2	8
51	13	4	17
52	11	6	17
53	11	2	13
54	14	2	16
55	14	2	16
56	19	7	26
57	14	12	26
58	28	14	42
59	24	6	30
60	13	1	14
61	13	3	16
62	9	—	9
63	8	1	9
64	2	—	2
65	1	1	2
66	6	—	6
67	3	—	3
68	—	—	—
69	—	—	—
70	1	—	1
Total	219	66	285

TABLE XI.

Number of A plants with 200 seeds or more in Crops J and K alone with each percentage of YR, YW, GR and GW seeds; together with the mean percentage M; the s.d., s; and the s.d. of sampling  $s_0$  based on the mean percentage observed.

Per-centage	No. of plants with said percentage of YR seeds	Per-centage	No. of plants with said percentage of YW seeds	No. of plants with said percentage of GR seeds	Per-centage	No. of plants with said percentage of GW seeds
47	1	12	1	1	2	—
48	—	13	—	1	3	1
49	—	14	—	2	4	8
50	2	15	3	3	5	7
51	3	16	5	6	6	14
52	5	17	8	9	7	13
53	2	18	8	8	8	9
54	1	19	9	7	9	3
55	1	20	10	8	10	1
56	6	21	4	4	—	—
57	10	22	5	3	—	—
58	14	23	1	2	—	—
59	6	24	1	2	—	—
60	1	25	—	—	—	—
61	3	26	—	—	—	—
62	—	27	1	—	—	—
63	—	—	—	—	—	—
64	—	—	—	—	—	—
65	1	—	—	—	—	—
Total	56	—	56	56	—	56
M	56.16	—	18.86	18.36	—	6.32
s	3.332	—	2.524	2.642	—	1.548
$s_0$	3.147	—	2.481	2.456	—	1.543
S.e. of M	.445	—	.332	.353	—	.207
S.e. of s	.315	—	.234	.250	—	.146

TABLE XII.

Showing for A plants of 200 seeds or more in Crops J and K alone, distinguishing plants with 54 per cent. of YR seeds or less from those with 55 per cent. or more, the numbers and proportions per 10,000 of each type of seed. For the totality of the seeds the value of P ( $\chi^2$  test) is .86.

Plants with percentages of YR's	Observed number of seeds				
	YR	YW	GR	GW	Total
47-54	1885	779	749	256	3669
55 upwards	6219	1932	1916	658	10725
Total	8104	2711	2665	914	14394
Expectation	8096.6	2698.9	2698.9	899.6	—
Distributions per 10000					
47-54	5138	2123	2041	698	10000
55 upwards	5799	1801	1786	614	10000
Total	5630	1883	1851	635	10000
Expectation	5625	1875	1875	625	10000



other, for when the proportion of YR's is high that of GW's is low and *vice versa*. On the contrary, the distributions are very nearly such as would be produced if the excess or defect in the proportion of YR's were distributed over the remaining types in proportion to the expected frequencies 3 : 3 : 1—i.e. such as we would get in random samples selected with the given proportions of YR's. Thus, in the lower group there is a deficiency in the proportion of YR's of 487 (5625-5138); distributing this in the proportions 3 : 3 : 1, we have to add to the remaining Mendelian proportions 209, 209 and 69 respectively, giving us expected totals of  $1875 + 209$ ,  $1875 + 209$  and  $625 + 69$ , or 2084, 2084, 694, which are very close to the observed figures 2123, 2041, 698. For the upper component I obtain similar expected proportions, by redistributing the deficiency in YR's, of 1800, 1800, 601, against the observed proportions 1801, 1786, 614. An examination of the data by the same method in much greater detail, taking separately the *A* plants with 47-50, 51-52, 53-55, 56, 57, 58, 59 and 60-65 per cent. of YR seeds, showed that all the samples obeyed this rule within the limits of random sampling. It would seem then that all the samples behave as random samples in a sense, but there are too many with a proportion of YR's round about 51-52 per cent. and correspondingly too many with a proportion round about 58 per cent. The effect is apparently of the kind that would be produced by differential mortalities falling on the seeds—but we should have to account for such differential mortalities just balancing out in the total!—and then of course we have still to account for the small standard deviations of the components. The whole matter remains to me an entire puzzle.

And yet the distribution for the entire 285 plants of Table VIII (A) suggests components with nearly the same means as those of the apparent components in the small sample of large plants. The maximum of the lower component looks as if it stood at about 51.5. There are 37 observations below this, which would make the total 74, but I judged it better to take a slightly lower figure as the tail of the upper component looked as if it would overlap, and made the figure tentatively 68. This would place the lower quartile at 50.125, suggesting a quartile deviation of 1.375 or a standard deviation of 2.04—say, roundly, two units. Extracting this component, assuming that it could be taken with sufficient precision as a normal curve, I then found that the upper component would be markedly skew owing to the high frequencies round 66 per cent. No adjustment of the lower component seemed to eliminate this difficulty and I consequently found it necessary to retain three components, the means and standard deviations of which were as follows:

	No.	Mean	S.D.
Lower component	68	51.5	2.00
Central       ,,	204	57.94	2.61
Upper         ,,	13	66	1.62

Owing to the tentative mode of procedure the three components superposed do not give precisely the same mean and s.d. as the observations, but the differences are quite small:

	Mean	S.D.
Observations	56.74	4.22
Fitted distribution	56.77	4.18

That the fit given is an excellent one can be seen from Fig. 8, p. 261, and the  $\chi^2$  test confirms the ocular impression. Grouping only the frequencies at 47 per cent. and under, 67 per cent. and over, so that  $n'$  is 21,  $\chi^2$  works out at 16.15, which makes  $P$  0.707, so that we might expect a worse fit seven times in ten. The signs of the deviations between calculated and observed frequencies are moreover well distributed, there being only one run of three differences of the same sign (from 53 to 55 per cent.), and these are both relatively and absolutely small.

The numbers of observations in the two principal components stand again in the incomprehensible ratio 3 : 1, and the whole distribution remains a complete puzzle.

If a more rigid test be made of the fit of the distributions for YW and GR seeds on the dihybrid plants by the method used above (p. 287), grouping the frequencies by the runs of sign of the differences between observation and theory, we obtain seven groups for the YW's,  $\chi^2$  comes to 10.63 and  $P$  is 0.10. For the GR's there are seven groups also,  $\chi^2$  is 7.91 and  $P$  0.25. The fit in the first case is poor, but there is little to suggest the difficulties that arise with the YR distribution. It is obvious that if the YR distribution be really compound, the other distributions for the dihybrid plants must also be compound, but it is quite possible that the two components might lie closer together and the compound distribution not be so suggestive of a double peak.

The distributions for the *B* and *C* plants tested in the same way give:

*B* plants (hybrids for shape): 10 groups;  $\chi^2$ , 12.07;  $P$ , 0.21.

*C* plants (hybrids for colour): 9 groups;  $\chi^2$ , 21.60;  $P$ , 0.0058.

For the second distribution  $P$  is very low indeed, and is strongly suggestive of something altogether abnormal. For the first  $P$  is quite moderate. The distribution for *C* plants is again rather suggestive of a compound distribution, but the components could not be taken as normal so I have not attempted dissection. I have not been able to throw any further light on these distributions.

*Summary.*

Summarising briefly the more important points that arise from the preceding discussion:

We have found that in five cases out of the six considered the observed standard deviations are greater than the standard deviations of sampling.

The frequency distribution for percentages of YR seeds on dihybrid plants is highly irregular and a very bad fit to the calculated distribution. It suggests a distribution with two components, centering round 51-52 per cent. of YR seeds and 58 per cent. respectively.

The large plants with 200 seeds or more in the two Crops *J* and *K*, the total seeds of which gave a close agreement with Mendelian expectation, nevertheless gave a distribution showing two peaks at about the same percentages, and suggesting two components with about the same relative numbers of seeds.

A difficulty is raised by the fact that, if there were two such components, their standard deviations would be very much lower than the standard deviations of sampling.

Whatever the explanation of the abnormal form of the YR-distribution may be, it seems impossible to account for it on the usual assumption that there is only a very simple mechanism at work. It suggests something more complex.

The distribution for percentages of Yellows on plants monohybrid for colour is also probably abnormal, and possibly compound.

#### V. THE SORTING OF ABNORMAL PLANTS BY THE PROPORTIONS OF THE FOUR TYPES OF SEED, AND AN INVESTIGATION OF ABNORMALITY BY LINES OF DESCENT.

In the preceding work the percentages of YR's, YW's, GR's and GW's on dihybrid plants have been considered singly, each percentage by itself. But, it may be asked, do any plants stand out as showing *combinations* of percentages of the four several types of seed diverging from the simple Mendelian proportions 9:3:3:1 by more than the fluctuations of sampling?

This question can only be answered by working out the value of  $\chi^2$  for each plant separately and looking up the value of *P*. The test has been applied to the plants with 100 seeds or more, but before going on to consider the results I should like to emphasise a point that is of importance. With the relatively small number of seeds that is borne by a single plant, hardly ever exceeding 300, we can only hope by this

process to sort out plants (if any) in which the true proportions diverge very largely indeed from 9 : 3 : 3 : 1. Thus, suppose that it was possible for gametes to be given off in the proportions 2 : 3 : 3 : 2 instead of 1 : 1 : 1 : 1, this would give phenotypes in the proportions 54 : 21 : 21 : 4. How many seeds would we require on a plant to make us—let us say—not even confident, but rather suspicious that we had not to deal with mere chance fluctuations from 9 : 3 : 3 : 1, or 5625 : 1875 : 1875 : 625? The value of  $\chi^2$  for a plant with 100 seeds would work out as follows:

54	56.25 - 2.25	.09
21	18.75 + 2.25	.27
21	18.75 + 2.25	.27
4	6.25 - 2.25	.81
		$\chi^2 = 1.44$

For this value of  $\chi^2$   $P$  is only 0.70, i.e. we would expect to get such a divergence from Mendelian expectation owing to fluctuations of sampling only, seven times in ten: we could lay no stress at all on the observed divergence. If the number of seeds on the plant is increased,  $\chi^2$  is increased in direct proportion; how many seeds will we then require in order to raise  $\chi^2$  to such a value that  $P$  will give rise to some suspicion? Suppose we take the limit (very moderately) at  $\chi^2 = 8$ , corresponding to  $P = 0.046$ , so that we might expect divergences more improbable than those observed, owing to random sampling only, about once in 21 trials. To give us this value of  $\chi^2$ , the number of observations (seeds on the plant) would have to be raised to  $100 \times 8/1.44$ , or 556. But no such plants occur; with plants of *Pisum* we are asking for a distinction that could not be made on the single plant.

One more point. Suppose that the true proportions were 54 : 21 : 21 : 4, as above, but that, owing to the chances of sampling, the plant with 100 seeds showed 51 : 23 : 23 : 2. This would at once raise the value of  $\chi^2$  for comparison with the Mendelian proportions to 5.13, or 8 with a plant of 156 seeds only. This suggests that if we sort out exceptional plants by the present method, they may tend to give us, not the true proportions of seeds that would be shown by plants of the type, but proportions diverging rather more largely, in the same direction, from the Mendelian proportions. We may tend to get, in fact, a biased sample.

With these warnings we may pass to Table XIII which shows the approximate frequency distribution of values of  $\chi^2$  for the 285 *A* plants with 100 seeds or more. It is only approximate, since a short process was used to save the labour of working out  $\chi^2$  from the numbers of seeds on the plants. The percentages of seeds had been already tabulated, and  $\chi^2$  was calculated from these percentages. Since a table could be drawn

TABLE XIII.

(A plants with 100 seeds or more) showing the numbers of plants observed with the rough value of  $\chi^2$  (as calculated from the percentages) within given limits in each crop, together with the totals for all the crops and the expected frequencies on random sampling.

Value of $\chi^2$	Crop						Totals	
	<i>G</i>	<i>H</i>	<i>J</i>	<i>K</i>	<i>L</i>	<i>M</i>	Observed	Expected
	Number of plants							
0—	1	4	16	19	4	12	56	56.7
1—	1	7	13	15	5	15	56	65.2
2—	4	3	15	21	9	12	64	51.5
3—	1	3	9	9	3 <sub>5</sub>	11	36	37.1
4—	1	2	8	6	2	3	22	25.6
5—	—	1	4	3	1	1	10	17.1
6—	—	—	3	1	3	3	10	11.4
7—	—	1	4	2	—	2	9	7.4
8—	—	—	1	3	—	3	7	4.8
9—	—	—	—	—	—	4	4	3.1
10 up.	—	1	3	3	—	4	11	5.3
Total	8	22	76	82	27	70	285	285.2

up giving once for all the value of  $\delta^2/m$ ,  $m$  being the theoretical frequency, for any observed percentage in either of the four classes, the work could be done very rapidly, adding up the entries in the table corresponding to the observed percentages and then multiplying the result by the actual number of seeds divided by 100. Rapid as it was, the process proved rather more rough in its results than I had realised it might be; in particular it tends to give too high a value of  $\chi^2$ . Such as it is, it will be seen that, looking to the column of totals on the right, the bulk of the distribution runs very closely with expectation, as deduced by differencing the column in the  $P$  table for  $n' = 4$  and multiplying by 285. But when we get to values of  $\chi^2$  over 7 there is a constant excess of observation over expectation. If we take  $\chi^2 = 8$  as the limit which may give rise to suspicion, there are 22 observed values of  $\chi^2$  exceeding this limit as against an expectation of 13 only. Later work showed that in three of these cases  $\chi^2$  as calculated from the seeds was rather under 8, but this leaves us with at least 19 (since re-working from the seed numbers might throw up a value under 8 to over 8) as against the expectation of 13. In the subsequent work, however, I have taken the 22 plants with *rough* values of  $\chi^2$  over 8 as "marked plants" for further investigation<sup>1</sup>.

The first thing done was to list all the dihybrid ( $A$ ) plants in each family with a "marked plant." The data are given in Table XIV, the "marked plant" of the family being distinguished by an asterisk against

<sup>1</sup> An examination of plants with less than 100 seeds would probably have contributed some "marked plants" but would have been very laborious. One such was noted.  $G$  22.1 gave 50 YR, 3 YW, 28 GR, 5 GW.  $\chi^2$  is 19.51 and  $P$  0.00022.



TABLE XIV (*continued*).

Numbers of seeds						Percentages				$\chi^2$	<i>P</i>
	YR	YW	GR	GW	Total	YR	YW	GR	GW		
<i>K</i> 10.2	85	38	38	7	168	51	23	23	4	4.81	.19
10.3	74	27	28	11	140	53	19	20	8	1.01	.80
10.4*	166	31	48	10	255	65	12	19	4	11.65	.0089
10.5	37	14	18	10	79	47	18	23	13	7.27	.065
Total	362	110	132	38	642	56	17	21	6	2.13 24.74	.55 .016
<i>K</i> 19.3*	89	39	30	19	177	50	22	17	11	8.07	.045
19.4	82	30	41	9	162	51	19	25	6	4.74	.20
19.5*	55	35	22	10	122	45	29	18	8	9.89	.020
19.7	99	40	37	11	187	53	21	20	6	1.19	.76
19.9	112	32	37	18	199	56	16	19	9	3.28	.36
19.10	127	49	46	19	241	53	20	19	8	1.89	.60
Total	564	225	213	86	1088	52	21	20	8	11.08 29.06	.011 .048
<i>K</i> 25.1*	74	31	37	2	144	51	22	26	1	10.33	.016
25.5	82	28	20	9	139	59	20	14	6	1.76	.63
25.9	129	47	36	18	230	56	20	16	8	2.42	.50
Total	285	106	93	29	513	56	21	18	6	1.45 14.51	.70 .11
<i>K</i> 27.2*	129	47	27	21	224	58	21	12	9	9.53	.024
27.8	44	8	16	5	73	60	11	22	7	2.99	.39
27.10	72	22	22	12	128	56	17	17	9	2.34	.51
Total	245	77	65	38	425	58	18	15	9	7.84 14.86	.050 .095
<i>M</i> 2.2	50	24	13	6	93	54	26	14	6	3.72	.30
2.3	82	31	30	8	151	54	21	20	5	0.67	.87
2.4	90	26	31	11	158	57	16	20	7	0.64	.87
2.5*	73	35	16	3	127	57	28	13	2	10.91	.012
2.6	112	39	30	11	192	58	20	16	6	1.48	.69
2.7	81	37	32	6	156	52	24	21	4	4.27	.24
2.9	42	16	9	3	70	60	23	13	4	2.54	.47
Total	530	208	161	48	947	56	22	17	5	8.88 24.23	.031 .28
<i>M</i> 12.3*	118	50	46	5	219	54	23	21	2	8.25	.042
12.4	31	5	14	3	53	58	9	26	6	4.21	.24
12.5	51	7	8	2	68	75	10	12	3	9.80	.021
12.7*	92	17	28	2	139	66	12	20	1	10.91	.012
12.8	70	28	15	3	116	60	24	13	3	6.73	.083
12.9	72	25	31	5	133	54	19	23	4	2.90	.41
Total	434	132	142	20	728	60	18	20	3	16.13 42.80	.0011 .0015
<i>M</i> 14.1*	76	30	11	9	126	60	24	9	7	8.99	.029
14.3	11	6	4	1	22	50	27	18	5	1.15	.77
14.4	26	6	6	2	40	65	15	15	5	1.24	.75
14.5	119	28	34	10	191	62	15	18	5	3.34	.35
14.6*	100	25	20	5	150	67	17	13	3	7.61	.056
14.7*	78	25	15	1	119	66	21	13	1	10.10	.018
Total	410	120	90	28	648	63	19	14	4	17.73 32.43	.00051 .029

TABLE XIV (continued).

	Numbers of seeds					Percentages				$\chi^2$	$P$
	YR	YW	GR	GW	Total	YR	YW	GR	GW		
<i>M</i> 22.1	37	12	15	3	67	55	18	22	4	.84	.83
22.3	48	18	24	1	91	53	20	26	1	6.91	.075
22.7	82	26	27	5	140	59	19	19	4	1.76	.63
22.10*	57	33	21	4	115	50	29	18	3	8.38	.040
Total	224	89	87	13	413	54	22	21	3	9.58 17.89	.023 .12
<i>M</i> 24.2	68	24	20	6	118	58	20	17	5	0.66	.13
24.3*	128	80	50	10	268	48	30	19	4	23.76	.000029
24.8	45	14	14	3	76	59	18	18	4	0.77	.85
24.10	158	61	69	14	302	52	20	23	5	5.16	.16
Total	399	179	153	33	764	52	23	20	4	16.34 30.35	.00099 .0027
<i>M</i> 26.2	31	15	17	2	65	48	23	26	3	4.44	.22
26.5*	80	35	15	6	136	59	26	11	4	8.76	.033
26.6	93	34	22	5	154	60	22	14	3	5.22	.16
26.7	60	19	25	6	110	55	17	23	5	1.24	.53
26.8*	62	36	26	5	129	48	28	20	4	8.62	.036
26.9*	85	15	17	4	121	70	12	14	3	9.94	.019
Total	411	154	122	28	715	57	22	17	4	10.47 38.22	.015 .0084

the record number on the left. On the right are given the values of  $\chi^2$  and of  $P$  for each plant. At the foot of the table for each family is given the total of the seeds for that family, and the percentages, and on the right the values of  $\chi^2$  and of  $P$  for this total. Immediately underneath these figures are given (1) the sum of the values of  $\chi^2$  for the individual plants of the family, and (2) the value of  $P$  found by taking  $n'$  as 1 more than three times the number of plants in the family. This value of  $P$  is the probability of obtaining on random sampling a collection of  $\chi^2$ 's as improbable as or more improbable than that observed. Both these tests seem necessary, for a family may diverge from expectation in two ways: (a) no one plant may diverge very largely from Mendelian expectation, but all the plants may diverge in the same direction, which will make the first value of  $P$  low, but the second possibly quite high; (b) the several plants may diverge considerably from Mendelian expectation, but in different directions, so that in the total for the family these divergences average out, which will make the first value of  $P$  possibly high, but the second low. A family must be taken as improbable for which *either* value of  $P$  is low.

It will be seen from Table XIV that the 22 marked plants fell into 16 families. Of these families not all would be judged to be improbable divergences from Mendelian expectation when considered as a whole. Taking  $P = .046$ , as before, as our moderate limit, only 10 of the 16



families show either  $P_1$  (from the total seeds in the family) or  $P_2$  (from the sum of the  $\chi^2$ 's) less than this value, but some of them, notably  $M$  12,  $M$  14 and  $M$  24, are highly improbable aggregates. In these three families  $P_1$  is in each case less than  $P_2$ , so that a certain tendency to similarity amongst the plants of the family is indicated, but this is by no means true of all the families: in eight of the 16 families  $P_1$  is greater than  $P_2$  and in eight it is less.

Considering the improbability of a "marked plant," the occurrence of two or three marked plants in one family is a striking feature. In Crop  $M$  there were 11 "marked plants" out of a total of 134 plants in 31 families, the numbers of dihybrid plants in these families being as follows:

Dihybrids in family	Number of families
1	1
2	3
3	5
4	9
5	4
6	7
7	2
Total	31

With these data, taking the chance of occurrence of a marked plant in this crop as  $11/134$  or  $0.082$ , we may calculate the expected numbers of families with 1, 2, 3, ... marked plants if the distribution of "marking" were random, assuming that the probabilities of 1, 2, 3, ... markings in a family of  $n$  will be given by the terms of  $(.918 + .082)^n$ . The results of the calculation were as follows:

Number of marked plants	Number of families	
	Calculated	Actual
0	21.61	25
1	7.94	3
2	1.31	1
3	.12	2
Over	.02	—
Total	31.00	31

If distribution of marking were random we should therefore have eight families with one "marked plant," whereas we only find three, and we would not expect a single family with three "marked plants," whereas we find two. The result suggests that, whatever the nature of these exceptional plants may be, the exceptional character is determined by something common to the family and probably heritable.

Much time was spent on an examination of the data of Table XIV to see whether any grouping round particular ratios other than the Mendelian proportions would suggest itself, but nothing resulted. In view of the

warnings already given, to obtain any certain result would clearly be difficult. "Marking" occurs rather more often with an excess than with a deficiency of YR's, and at first two contrasted types suggested themselves as occurring rather frequently, characterised by an excess of YR's combined with an excess of either YW's or GR's. The following will serve as examples:

Plant	Percentages of seeds			
	YR	YW	GR	GW
<i>K</i> 9.9	59	23	13	5
<i>M</i> 2.5	57	28	13	2
<i>M</i> 14.1	60	24	9	7
<i>M</i> 14.7	66	21	13	1
<i>J</i> 2.10	61	11	23	5
<i>J</i> 25.5	67	9	19	4
<i>K</i> 10.4	65	12	19	4
<i>M</i> 12.7	66	12	20	1

The above are all "marked plants," but some of the unmarked plants—plants with too few seeds to make their exceptional proportions highly improbable—seem to fall into one or other of the two groups fairly closely, the first group characterised by about 84 yellows to 16 greens, the second by about 84 rounds to 16 wrinkleds, the other ratio in each case being nearly normal. A long and tedious examination of the whole mass of available data made me doubt however whether such proportions did really represent definite genetic types; I failed to convince myself, for example, that a high percentage of YR's was at all exceptionally associated with very unequal proportions of YW's and GR's.

But the conclusion that the incidence of the "marking" on particular families suggests something heritable renders an examination of the offspring of "marked plants" desirable. Unfortunately such an examination almost breaks down at the outset. To begin with, 11 of the 22 "marked plants" occur in Crop *M*, and owing to the failure of the following crops and the cessation of the experiment no offspring of any of the plants of that generation are available. Secondly, owing to the method of conducting the experiment, ten yellow-round seeds being sown from each of some 30 or 40 dihybrid plants only out of about 130, the majority of dihybrid plants in each generation leave no progeny, and those that do leave some leave so few that it is difficult to base any judgment on the data.

On searching the records it was found that seeds were sown from three only of the 11 marked plants in generations *G* to *L*, viz. *H* 39.1, *J* 25.5 and *K* 19.3. All the descendants of these three plants are shown in Table XV (A), (B) and (C). The percentage distribution of the seeds of the parent plant is given as a reminder at the head of each section of the table; particulars of the actual numbers of seeds of the parent will be found in Table XIV.

*H* 39-1 was a plant with a small excess of YR's and a considerable excess of YW's—an excess in fact of both types of yellow; yellows to greens are in the proportions 83 : 17, rounds to wrinkleds in the proportions 72 : 28. As will be seen from Table XIV (A), another of the

TABLE XV.

*Showing the recorded descendants of certain "marked plants," having a rough value of  $\chi^2$  (calculated from the percentages) of 8 or over.*

Record number	Numbers of seeds					Percentages			
	YR	YW	GR	GW	Total	YR	YW	GR	GW
<i>J</i> 29 from <i>H</i> 39-1									
29-2	45	13	14	3	75	60	17	19	4
29-4*	133	78	54	20	285	47	27	19	7
29-5	161	47	—	—	208	77	23	—	—
29-3	77	—	31	—	108	71	—	29	—
29-6	160	—	47	—	207	77	—	23	—
29-7	58	—	14	—	72	81	—	19	—
29-1	52	—	—	—	52	100	—	—	—
29-8	62	—	—	—	62	100	—	—	—
<i>K</i> 24 from <i>J</i> 29-2									
24-2	41	11	14	4	70	59	16	20	6
24-3	39	16	18	2	75	52	21	24	3
24-4	103	31	24	6	164	63	19	15	4
24-5	105	43	42	16	206	51	21	20	8
24-8	50	18	19	10	97	52	19	20	10
24-9	125	32	41	13	211	59	15	19	6
24-10	84	29	34	9	156	54	19	22	6
24-1	75	—	23	—	98	77	—	23	—
24-6	23	—	9	—	32	72	—	28	—
24-7	64	—	—	—	64	100	—	—	—
<i>L</i> 10 from <i>K</i> 24-3									
10-6	59	10	20	6	95	62	11	21	6
10-1	52	16	—	—	68	76	24	—	—
10-5	44	14	—	—	58	76	24	—	—
10-8	31	19	—	—	50	62	38	—	—
10-10	54	20	—	—	74	73	27	—	—
10-3	6	—	6	—	12	50	—	50	—
10-4	61	—	33	—	94	65	—	35	—
10-7	37	—	13	—	50	74	—	26	—
10-2	70	—	—	—	70	100	—	—	—
10-9	36	—	—	—	36	100	—	—	—
<i>M</i> 8 from <i>L</i> 10-6									
8-3	85	31	35	13	164	52	19	21	8
8-4	22	12	6	5	45	49	27	13	11
8-5	83	36	30	8	157	53	23	19	5
8-10	61	28	25	7	121	50	23	21	6
8-2	53	14	—	—	67	79	21	—	—
8-9	73	29	—	—	102	72	28	—	—
8-6	59	—	22	—	81	73	—	27	—
8-1	91	—	—	—	91	100	—	—	—
8-7	88	—	—	—	88	100	—	—	—
8-8	99	—	—	—	99	100	—	—	—

TABLE XV (continued)

(B) K 28 from J 25.5 (67:9:19:4)

Record number	Numbers of seeds					Percentages			
	YR	YW	GR	GW	Total	YR	YW	GR	GW
28.3	20	3	5	1	29	69	10	17	3
28.10	175	52	60	21	308	57	17	19	7
28.1	52	17	—	—	69	75	25	—	—
28.6	23	4	—	—	27	85	15	—	—
28.7	106	37	—	—	143	74	26	—	—
28.4	14	—	10	—	24	58	—	42	—
28.5	51	—	18	—	69	74	—	26	—
28.8	37	—	8	—	45	82	—	18	—
28.9	94	—	—	—	94	100	—	—	—

L 6 from K 28.3

6.5	9	5	2	1	17	53	29	12	6
6.6	60	26	21	10	117	51	22	18	9
6.8	65	22	17	3	107	61	21	16	3
6.9	78	26	18	10	132	59	20	14	8
6.10	14	4	7	—	25	56	16	28	—
6.2	71	17	—	—	88	81	19	—	—
6.4	13	2	—	—	15	87	13	—	—
6.7	51	17	—	—	68	75	25	—	—
6.1	56	—	21	—	77	73	—	27	—
6.3	21	—	—	—	21	100	—	—	—

M 12 from L 6.6

12.3*	118	50	46	5	219	54	23	21	2
12.4	31	5	14	3	53	58	9	26	6
12.5	51	7	8	2	68	75	10	12	3
12.7*	92	17	28	2	139	66	12	20	1
12.8	70	28	15	3	116	60	24	13	3
12.9	72	25	31	5	133	54	19	23	4
12.1	63	16	—	—	79	80	20	—	—
12.2	16	10	—	—	26	62	38	—	—
12.6	136	59	—	—	195	70	30	—	—
12.10	63	—	18	—	81	78	—	22	—

(C) L 14 from K 19.3 (50:22:17:11)

Record number	Numbers of seeds					Percentages			
	YR	YW	GR	GW	Total	YR	YW	GR	GW
14.1	19	12	7	2	40	47	30	18	5
14.6	72	22	24	5	123	59	18	20	4
14.8	50	14	9	5	78	64	18	12	6
14.9	57	13	11	1	82	70	16	13	1
14.2	36	12	—	—	48	75	25	—	—
14.10	48	19	—	—	67	72	28	—	—
14.3	13	—	4	—	17	76	—	24	—
14.4	25	—	10	—	35	71	—	29	—
14.5	57	—	14	—	71	80	—	20	—
14.7	22	—	—	—	22	100	—	—	—

M 4 from L 14.1

4.5	64	23	26	3	116	55	20	22	3
4.6	28	7	11	1	47	60	15	23	2
4.9	74	27	18	6	125	59	22	14	5
4.3	33	11	—	—	44	75	25	—	—
4.4	57	—	12	—	69	83	—	17	—
4.7	54	—	9	—	63	86	—	14	—
4.8	81	—	30	—	111	73	—	27	—
4.1	59	—	—	—	59	100	—	—	—
4.2	76	—	—	—	76	100	—	—	—

marked plants, *J* 29·4, occurred in the immediately following generation, but this plant showed a heavy deficiency of YR's accompanying the excess of YW's; the proportion of yellows to greens is 74 : 26, or almost in accordance with Mendelian expectation, but the proportion of rounds to wrinkleds is 66 : 34. Of the seven dihybrid plants in the next generation, *K* 24·4 suggests a resemblance to the original ancestor, the proportion of yellows to greens being 82 : 19 and of rounds to wrinkleds 78 : 23. The other plants are not very abnormal. Crop *L* gave only one dihybrid with no more than 95 seeds, yellows to greens being 73 : 27, and rounds to wrinkleds 83 : 17. In *M* 8 all the dihybrids show a deficiency of YR's and *M* 8·4 is very reminiscent of *J* 29·4; yellows to greens are 76 : 24 and rounds to wrinkleds 62 : 38. So far as it goes—and it does not go very far in view of the small numbers of seeds on many of the plants—this suggests that in the progeny of a "marked plant" we must not look only for a tendency to resemble the proportions given by that plant, but to a breaking up into plants with other abnormal ratios; we have in this pedigree two quite different abnormal proportions, *H* 39·1 and *J* 29·4, a possible reciprocal of the *H* 39·1 type in which the proportions of yellows to greens and rounds to wrinkleds are interchanged, and normal ratios, or ratios concerning which we would hardly be suspicious.

The other pedigrees, it seems to me, give something of the same sort of picture. *J* 25·5 shows a marked excess of YR's and a slight excess of GR's; yellows to greens are 76 : 23, rounds to wrinkleds 86 : 13. In the following generation there are only two dihybrid plants, one of which has only 29 seeds. So far as it goes the distribution of seeds on this plant is more like that of the parent than the Mendelian distribution, but one can hardly lay much stress on the likeness in view of the small number of seeds;  $\chi^2$  from Mendelian expectation would work out at about 2·29, from the distribution of the parent plant at about 0·16. The second dihybrid, a big plant with over 300 seeds, gave a distribution very close to 9 : 3 : 3 : 1 expectation. In *L* 6 all the divergences are well within the limits of fluctuations of sampling. In *M* 12 there are two marked plants again, one of which, *M* 12·7, rather resembles the ancestor *J* 25·5, showing yellows to greens 78 : 21, rounds to wrinkleds 86 : 13, the other being quite different and giving both yellows to greens and rounds to wrinkleds near 3 : 1, but YR's and GW's in defect. It is one of the few marked distributions suggestive of coupling or repulsion.

Finally, turning to Table XIV (c), we have the descendants of a plant one of the principal characteristics of which is a marked excess, instead of the usual deficiency, of GW seeds, YR seeds being in defect; yellows

are to greens in the proportion 72 : 28, rounds to wrinkleds in the proportion 67 : 33. The progeny in *L* 14 are a poor lot with few seeds per plant. So far as they go *L* 14.1 most nearly resembles the parent, though it has no excess of GW seeds, yellows to greens being 77 : 23 and rounds to wrinkleds 65 : 35. The other three plants all show an excess of both yellows and rounds. The three dihybrids of *M* 4 are again almost featureless; no one of them suggests the ancestral type of distribution, nor anything abnormal beyond the deficiency of GW seeds; the ancestral excess of GW seeds does not reappear.

I have given in the table the monohybrid as well as the dihybrid plants descended from each "marked plant," but the monohybrids hardly suggest anything abnormal. Nor are the numbers of the different types of plant (dihybrids, monohybrids for shape and for colour, and pure dominants) sufficiently large for one to be able to lay much stress on their divergences.

Having regard to the small amount of evidence available respecting the progeny of "marked plants," it occurred to me that some further evidence as to the heritability or otherwise of a tendency to throw plants with abnormal ratios might be obtained by separating the records for different lines of descent. It is true that, as suggested by our examination of Table XV, plants in an "abnormal line" (if there is such a thing) may be thrown with different abnormal ratios that may more or less compensate in the total, but on the other hand, they may *not* compensate and the investigation seemed at least worth while. The ascendants of each family in Crop *M* were therefore traced through the sowing record and data so obtained for 29 separate lines of descent; two lines were omitted owing to errors in the sowing record, which rendered it impossible to carry the data for them back to Crop *G*, and their partial inclusion was hardly thought worth while. The lines have been denoted by the number of the final family in Crop *M*, but it will be understood that each line is the aggregate of descendants of one plant in Crop *F*. Owing to a breach in the records it is not possible to carry back the genealogy of each line to the beginning, but the following sets of lines have each a common ancestor:

3 and 4 with a common ancestor in *C*.

12 and 14 with a common ancestor in *C*.

17 and 18 with a common ancestor in *C*.

13, 15 and 16; 15 and 16 having a common ancestor in *F* and 13 a common ancestor with the others in *C*.

Table XVI gives the data for the seeds of the dihybrid plants in the 29 lines. The numbers of the seeds in each line are given first, then these

numbers reduced to proportions per 10,000 of the total in the line, and finally in the columns on the right two values of  $P$ . The first gives the probability of obtaining an equal or worse fit to Mendelian expectation on random sampling from a Mendelian population, and is obtained straightforwardly by working out  $\chi^2$  from the Mendelian expectation and entering the tables with  $n'$  as 4. The second gives the probability of obtaining a worse fit than that shown to the average *observed* distribution given by the total of the seeds at the foot of the table, and is obtained by working out  $\chi^2$  for a two-row table consisting of the given line and the aggregate of the remaining lines, and entering the tables with  $n' = 4$  again. Table XVII gives data in the same form for the number of plants of each type:  $A$ , dihybrids,  $B$ , hybrids for shape,  $C$ , hybrids for colour, and  $D$ , pure dominants.

Inspection of these tables will at once suggest that the 29 lines cannot be regarded as random selections either from a Mendelian population or from a population with the average constitution of the group. Taking Table XVI and the first column for  $P$ , it will be seen that seeds in the proportions occurring in line 14 would only be expected on random sampling some three times in 10,000 trials; in line 1 about twice in 1000 trials; in line 24 about five times in 1000 trials. No one of these lines can well be regarded as a random deviation from Mendelian proportions. At the same time it is a striking fact that a number of the lines give quite good fits to Mendelian expectation, notably lines 28, 20 and 21. Taking the second column for  $P$ , giving the probability of divergence from the average distribution, neither line 14 nor line 1 can well be regarded as a random divergence from the average. Much the same sort of thing will be noted in the following table for the different types of plant, but the values of  $P$  in the two tables do not run very closely together. Line 28, for example, gives an extraordinarily close fit to Mendelian expectation for seeds, but a poor fit for the different types of plant. Line 14, which gives such an impossible fit to Mendelian expectation for seeds, gives quite a fair fit for the different types of plant.

To bring the two pieces of information together and enable a better judgment to be formed respecting each line on the basis of both seeds and plants, I have given in Table XVIII the values of  $P$  for seeds and plants jointly; the values of  $\chi^2$  for seeds and for plants respectively, in the same line, are added together and the tables entered with  $n' = 7$ . This table gives, but more clearly, the same impression as before. The first column shows definitely that some lines are not Mendelian in their proportions of seeds and plants, but others very well may be. The second column shows that the odds are over 200 to 1 against line 14 being a random

deviation from the average, and nearly 100 to 1 against line 1; moreover, while we should only expect a single line, with random sampling, to give a value of  $P$  less than  $1/29$  or 0.0345, there are actually four such lines.

Table XIX summarises the matter further, the numbers of values of  $P$  between the limits 0 and  $5/29$ ,  $5/29$  and  $10/29$ , and so on up to  $25/29$  to 1 being counted up in each of the Tables XVI, XVII and XVIII (using values of  $P$  calculated to more digits than those given). On random sampling the values of  $P$  should be uniformly distributed between 0 and 1, and consequently we should expect five values in each of the groups up to the last, where there should be four. The observed distributions are very different; there is always an excessive number of low values of  $P$ —9 to 14 against the expected 5—and if the two highest groups are taken together there is always a deficiency of high values—4 to 7 against the expected 9. Testing these distributions against expectation by the  $\chi^2$  method, we find for the first three distributions low, but not at all impossible, values for  $P$ , but for both the last two very low values. A further test may be applied by a method which is, so far as I know, novel but appears to me useful and legitimate. If we had to deal with a case of pure random sampling the values of  $P$  should be uniformly scattered over the range 0 to 1, and the mean value of  $P$  should therefore be 0.5, its standard deviation  $\sqrt{1/12}$  or 0.2887. The standard error of the mean of  $P$  in samples of 29 is consequently  $0.2887/\sqrt{29}$  or 0.0536. Taking the average values of  $P$  in the different cases<sup>1</sup>, we find results as follows:

Case	Mean of $P$	Difference from 0.5	Difference over s.e.	Chance of greater difference in defect
Seeds from Mendelian	.3555	.1445	2.69	.0036
Seeds from average	.3746	.1254	2.34	.0096
Plants from Mendelian	.3764	.1236	2.31	.010
Plants from average	.4260	.0740	1.40	.081
Seeds and plants:				
From Mendelian	.3206	.1794	3.35	.00040
From average	.3598	.1402	2.62	.0044

The figures are quite confirmatory of the previous conclusion. Considering seeds and plants together, the average value of  $P$  is less than 0.5 by 3.35 times its standard error when we consider the deviations from Mendelian expectation, and 2.62 times its standard error when we consider deviations from the average distributions. I think it may be concluded with certainty that, considered as a whole, these lines are not only non-Mendelian in their behaviour, but they do not form a homogeneous group.

<sup>1</sup> As above, these averages were determined on values carried to more significant figures than those given.



If the group is not homogeneous, the question naturally arises whether it can be sorted into homogeneous sub-groups. In particular, can any number of lines be picked out which may be regarded as normal Mendelian lines both as regards the proportions of seeds and of different types of plant?

I have made such an attempt at sorting, based partly on the values of  $P$  given in Tables XVI to XVIII, and partly on values of  $\chi^2$  reduced to a constant number of observations, so as to give a test of absolute fit (instead of fit in terms of the chances of sampling) to Mendelian expectation or to the average distribution as the case might be. In this way I

TABLE XVI.

*Showing the numbers and proportions per 10,000 of seeds on A plants (dihybrids) in each of 29 lines, each line including only descendants of a single plant in Crop F, but denoted by the number of the final family in M. On the right are given the probabilities of occurrence of the observed divergence on random sampling (a) from a Mendelian distribution, (b) from the distribution given by the totality of the seeds in these lines.*

Line: ascendants of M	Numbers of seeds					Proportions per 10,000				P	
	YR	YW	GR	GW	Total	YR	YW	GR	GW	From Mendelian expectation	From average distribution
1	976	371	294	84	1725	5657	2151	1704	487	·0019	·0093
3	1256	446	432	118	2252	5577	1980	1918	524	·15	·38
4	1285	461	454	162	2362	5440	1952	1922	686	·28	·062
5	469	177	170	48	864	5428	2049	1968	556	·39	·47
6	969	350	300	107	1726	5614	2028	1738	620	·28	·30
7	1424	435	431	125	2415	5896	1801	1785	518	·025	·10
8	1324	500	475	154	2453	5397	2038	1936	628	·10	·057
9	1186	419	421	147	2173	5458	1928	1937	676	·43	·15
11	1674	566	558	209	3007	5567	1882	1856	695	·46	·067
12	1249	373	381	108	2111	5917	1767	1805	512	·025	·084
13	894	289	314	82	1579	5662	1830	1989	519	·25	·42
14	801	230	194	71	1296	6181	1775	1497	548	·00029	·00068
15	1398	504	453	152	2507	5576	2010	1807	606	·35	·40
16	1389	460	456	136	2441	5690	1884	1868	557	·57	·93
17	1345	440	421	145	2351	5721	1872	1791	617	·73	·72
18	1086	371	341	107	1905	5701	1948	1790	562	·45	·78
19	926	294	321	97	1638	5653	1795	1960	592	·69	·64
20	1194	387	395	130	2106	5670	1838	1876	617	·95	·85
21	844	272	271	90	1477	5714	1842	1835	609	·90	·90
22	1380	445	445	125	2395	5762	1858	1858	522	·18	·53
23	1084	357	394	119	1954	5548	1827	2016	609	·46	·31
24	2272	811	750	205	4038	5627	2008	1857	508	·0052	·059
25	1656	558	550	160	2924	5663	1908	1881	547	·38	·84
26	1020	347	336	82	1785	5714	1944	1882	459	·037	·16
27	1893	561	639	203	3296	5743	1702	1939	616	·079	·034
28	2313	762	777	257	4109	5629	1854	1891	625	·97	·60
29	343	98	116	29	586	5853	1672	1980	495	·29	·38
30	1199	396	374	145	2114	5672	1873	1769	686	·46	·17
31	1334	412	448	135	2329	5728	1769	1924	580	·41	·46
Total	36183	12092	11911	3732	63918	5661	1892	1863	584	·00016	—

first picked out 16 lines which I thought might be regarded as normal Mendelian lines; obviously, let me say at once, any such selection is bound to be more or less arbitrary and two different observers or the same observer at two different times might not make quite the same selection. This first selection left me with 13 lines which I judged to be non-Mendelian in their behaviour, but which still did not appear to be homogeneous. A suggestion for further sub-division was given by noting that lines 1 and 3 appeared to be more or less alike, and also lines 14 and 29. Taking these two pairs of lines as a guide, the 13 non-Mendelian

TABLE XVII.

Table showing the numbers and proportions of plants of each type in the lines of Table XVI: A, dihybrid plants; B, hybrids for shape; C, hybrids for colour; D, pure dominants. The Mendelian expectation is 4:2:2:1 or in parts per 10000, 4445:2222:2222:1111. The probabilities P on the right are the probabilities of the observed divergence on random sampling, (a) from a Mendelian population, (b) from a population with the average observed distribution.

Line: ascend- ants of M	Numbers of each type of plant					Percentages (per 10,000 at foot)				P	
										From Mendelian expecta- tion	From average distribu- tion
	A	B	C	D	Total	A	B	C	D		
1	24	9	8	12	53	45	17	15	23	.042	.17
3	25	9	7	14	55	45	16	13	25	.0039	.032
4	22	8	14	12	56	39	14	25	21	.058	.29
5	18	11	12	6	47	38	23	26	13	.84	.92
6	18	13	16	7	54	33	24	30	13	.38	.54
7	24	12	12	8	56	43	21	21	14	.89	.98
8	23	9	11	12	55	42	16	20	22	.082	.34
9	19	13	12	12	56	34	23	23	21	.077	.35
11	24	12	15	4	55	44	22	27	7	.72	.50
12	22	19	12	4	57	39	33	21	7	.19	.095
13	20	10	13	12	55	36	18	24	22	.074	.37
14	17	13	13	8	51	33	25	25	16	.42	.67
15	20	9	12	7	48	42	19	25	15	.71	.96
16	27	8	16	5	56	48	14	29	9	.39	.29
17	23	11	12	4	50	46	22	24	8	.89	.66
18	20	19	10	8	57	35	33	18	14	.16	.14
19	22	8	16	8	54	41	15	30	15	.33	.54
20	29	13	11	3	56	52	23	20	5	.48	.18
21	19	12	14	12	57	33	21	25	21	.076	.37
22	23	14	9	8	54	43	26	17	15	.62	.66
23	22	14	14	7	57	39	25	25	12	.85	.87
24	31	9	10	7	57	54	16	18	12	.39	.26
25	24	12	16	7	59	41	20	27	12	.82	.87
26	21	16	7	10	54	39	30	13	19	.093	.15
27	31	8	11	5	55	56	15	20	9	.32	.15
28	34	7	17	2	60	57	12	28	3	.026	.0079
29	9	14	16	8	47	19	30	34	17	.0058	.016
30	21	10	15	7	53	40	19	28	13	.65	.82
31	27	14	14	2	57	47	25	25	4	.35	.14
Total	659	336	365	221	1581	4168	2125	2309	1398	.0014	—

TABLE XVIII.

Showing the values of  $P$ , or probabilities of obtaining on random sampling a divergence as great as or greater than that observed, (a) from a Mendelian population, (b) from a population corresponding to the averages observed, in the lines of Tables XVI and XVII, when the results for seeds and for types of plant are considered together.

Line: ascendants of $M$	Seeds and plants together $P$ from	
	Mendelian expectation	Average distribution
1	.00074	.011
3	.0046	.065
4	.078	.084
5	.70	.81
6	.32	.44
7	.13	.39
8	.04	.090
9	.14	.19
11	.68	.15
12	.028	.042
13	.086	.42
14	.0014	.0048
15	.58	.79
16	.54	.66
17	.93	.81
18	.24	.36
19	.55	.70
20	.83	.46
21	.29	.72
22	.35	.70
23	.76	.64
24	.015	.07
25	.67	.96
26	.02	.10
27	.10	.029
28	.15	.033
29	.01	.037
30	.64	.44
31	.40	.23

TABLE XIX.

Number of lines with a value of $P$ between the limits on the left							
Value of $P$	Expec- tation	Seeds from Mendelian expecta- tion	Seeds from average distrib- ution	Plants from Mendelian expecta- tion	Plants from average distrib- ution	Seeds and plants from Mendelian expecta- tion	Seeds and plants from average distrib- ution
0- 5/29	5	9	11	11	9	14	12
5/29-10/29	5	5	3	3	5	3	2
10/29-15/29	5	9	6	6	4	2	6
15/29-20/29	5	2	3	2	5	6	2
20/29-25/29	5	1	4	5	1	3	6
25/29- 1	4	3	2	2	5	1	1
Value of $\chi^2$	—	11.65	10.00	9.80	6.85	22.05	16.05
Value of $P$	—	.0406	.0752	.0820	.2335	.00051	.0067

lines were subdivided into two groups containing respectively 7 and 6 lines<sup>1</sup>. The classification was as follows:

Group  $\alpha$ . Approximately normal Mendelian. Lines 4, 5, 6, 7, 9, 11, 15, 16, 17, 19, 20, 23, 27, 28, 30, 31.

Group  $\beta$ . Lines 1, 3, 8, 22, 24, 25, 26.

Group  $\gamma$ . Lines 12, 13, 14, 18, 21, 29.

Group  $\alpha$ . This group gives totals of seeds and of plants as follows: as in Table XVII, *A* stands for dihybrid plants, *B*, hybrids for shape, *C*, hybrids for colour, *D*, pure dominants.

	Seeds					Plants				
	YR	YW	GR	GW	Total	A	B	C	D	Total
Numbers	21082	6981	7012	2317	37392	381	171	219	99	870
Proportions	5638	1867	1875	620	10000	438	197	252	114	1000

The totals of seeds give an excellent fit to Mendelian expectation (21033 : 7011 : 7011 : 2337);  $\chi^2$  works out at 0.41 and *P* at 0.92. The fit for the proportions of different types of plant is not so good, as the numbers of *B* and *C* plants are very unequal. Mendelian expectation is 386.7 : 193.3 : 193.3 : 96.7;  $\chi^2$  works out at 6.12 and *P* is 0.11. The fit, though poor, is therefore within the limits of fluctuations of sampling since a worse fit would be expected about once in nine trials. If the values of *P* for the individual lines are collected, it will be found that they are well scattered over the range 0 to 1, the averages being:

Seeds from Mendelian expectation	...	...	0.4710
Plants from Mendelian expectation	...	...	0.4970
Seeds and plants from Mendelian expectation	...	...	0.4708

These results seem very fair. If we had been given these 16 lines alone, I do not think any question would have been raised respecting their purely Mendelian behaviour. The only possible question would have been suggested by the excess of *C* plants in the majority of the lines (11 out of 16) and in the total.

Group  $\beta$ . The totals of seeds and of plants in this group are:

	Seeds					Plants				
	YR	YW	GR	GW	Total	A	B	C	D	Total
Numbers	9884	3478	3282	928	17572	171	78	68	70	387
Proportions	5625	1979	1868	528	10000	442	202	176	181	1000

In this group the most striking features are the deficiency of GW seeds, 5.28 per cent. instead of 6.25 per cent. or 928 seeds instead of 1098;

<sup>1</sup> I suppose it will strike the reader that 7 : 16 : 6 is very near 1 : 2 : 1, but what meaning can be attached to such proportions?

and the excess of  $D$  plants, 18.1 per cent. instead of 11.1 per cent. or 70 plants instead of 43.

Admitting the group as non-Mendelian, we have still to enquire whether it can be regarded as homogeneous. The test was applied by working out the value of  $\chi^2$  for each line from the average, forming a two-row table with the given line as one row and the total of the remaining rows as the other, as before, and taking  $n'$  as 4. For seeds and plants together, the two separate values of  $\chi^2$  were added and the tables entered with  $n' = 7$ . The values of  $P$  for the several lines are as follows:

Line	Seeds	Plants	Seeds and Plants
1	.1025	.7463	.2820
3	.9086	.3461	.7018
8	.0267	.7395	.1062
22	.3903	.6937	.6129
24	.8688	.3357	.6647
25	.7395	.1555	.3679
26	.5363	.2667	.4068
Mean	.5104	.4691	.4489

These results seem to me very satisfactory. With only seven lines the standard error of the mean value of  $P$  would be 0.109, and the deviations from the expected mean, 0.5, are no more than .0104, .0309 and .0511. The group can, apparently at least, be regarded as a homogeneous group.

Group  $\gamma$ . The totals of seeds and of plants are:

	Seeds					Plants				
	YR	YW	GR	GW	Total	A	B	C	D	Total
Numbers	5217	1653	1617	487	8954	107	87	78	52	324
Proportions	5826	1824	1806	544	10000	330	269	241	160	1000

The most marked feature in the seeds of this group is again the deficiency of GW's, of which there are only 5.44 per cent. instead of 6.25 per cent. or 487 seeds instead of 560; but there is also an excess of YR's, 58.26 per cent. instead of 56.25 per cent., or 5217 seeds instead of 5037. In the case of the plants, there is a marked deficiency of  $A$  plants, 33 per cent. instead of 44.4 per cent. or 107 plants instead of 144; and an excess of  $D$  plants, though not so great as in the last group, 16 per cent. instead of the Mendelian expectation of 11.1 per cent. or 52 plants instead of 36.

Testing the group for homogeneity as in the case of group  $\beta$ , I find the following values of  $P$  for the deviations from the average:

Line	Seeds	Plants	Seeds and Plants
12	.7120	.1441	.3362
13	.2041	.3265	.2291
14	.0097	.9805	.0736
18	.4169	.4730	.4944
21	.5976	.5747	.6925
29	.5489	.1297	.2522
Mean	.4149	.4381	.3463

These results are by no means so good as those for group  $\beta$ . The chance that a worse fit to the average than that given by the seeds of the remarkable line 14 would arise on random sampling is less than 1 in 100, and the average value for  $P$  when seeds and plants are considered together is only 0.3463. As the standard error for the mean value of  $P$  with six lines would be 0.118, this is a deviation from 0.5 of 1.30 times the standard error, which would only occur on random sampling in one trial out of ten. No value of  $P$  is so very low as absolutely to exclude the possibility of homogeneity, but the fit is not good.

It may be mentioned that of the 22 "marked plants," one falls into an omitted line, seven fall into group  $\alpha$ , eight into group  $\beta$  and six into group  $\gamma$ . The aberrant groups contain two-thirds of the "marked plants," and both families containing three "marked plants."  $G$  22.1, the "marked plant" with less than 100 seeds mentioned in the footnote on p. 295, falls into line 27 in group  $\alpha$ .

I do not want to lay much stress on the particular grouping that I have effected in this way; as already stated, it is necessarily more or less arbitrary and different attempts would almost certainly lead to slightly different results. But it serves to bring out, as I think no other process does, two points that seem to me important. The first is that quite a number of the lines give figures that do not differ significantly from simple Mendelian expectation. It follows that the divergences from Mendelian expectation noted in the totals of Table VI (A), p. 273, and Table I, p. 264, for the seeds and plants of all the six crops together are *mainly* contributed from certain particular lines.

Thus, in Table VI (A) the main features in the total are an excess of 242 YR seeds and a deficiency of 309 GW seeds. The six lines of group  $\gamma$  alone contribute no less than 180 to the excess of YR's although they contain only 8954 seeds out of the 73,187 of Table VI (A); roundly, that is, they contribute three-quarters of the excess from one-eighth of the seeds. Again, groups  $\beta$  and  $\gamma$  together contribute 243 to the deficiency of 309 GW seeds, although they contain together only 26,526 seeds; roundly, that is, they contribute 80 per cent. of the deficiency from 35 per cent. of the seeds.

Again, in Table I the main features noted in the total at the foot were a deficiency of 75  $A$  plants and an excess of 54  $D$  plants. Group  $\gamma$  contributes 37 to the deficiency of  $A$  plants, although these six lines have only 324 out of the 1913 plants of Table I; that is to say, 50 per cent. of the deficiency is contributed by lines with only 17 per cent. of the plants. Groups  $\beta$  and  $\gamma$  together contribute 43 to the excess of 54  $D$  plants, although they contain only 711 plants of the 1913; that is to say, 80 per

cent. of the deficiency is contributed by lines with only 37 per cent. of the plants. This seems clearly to indicate, as already stated in Section II (pp. 267, 270), that the divergences from expectation in the proportions of plants are in some way genetic in origin.

The second point to be noted is that it seems very difficult to relate the proportions of seeds to the proportions of plants in the same line or group. The difficulty was suggested by Tables XVI and XVII, but the number of plants in the single line is so small that fluctuations of sampling are sufficient to render any result obscure. With the groups the matter comes out very clearly. Groups  $\beta$  and  $\gamma$  differ in the proportions of their seeds, significantly indeed, for arranging the data as a two-row table  $\chi^2$  comes to 13.19,  $n'$  is 4, and  $P$  is 0.0043 only; but the difference is actually not large for the proportions are:

	YR	YW	GR	GW
Group $\beta$	5625	1979	1868	528
„ $\gamma$	5826	1824	1806	544

But the proportions of the different types of plant given by the YR seeds from dihybrid plants differ in quite a striking fashion:

	A	B	C	D
Group $\beta$	442	202	176	181
„ $\gamma$	330	269	241	160

A genetic analysis of the ovules and pollen on exceptional plants of the kind might throw some light on the matter; statistically it remains another puzzle.

While the groups given, or at all events groups  $\alpha$  and  $\beta$ , may be homogeneous groups of lines, it by no means follows that the individual lines are homogeneous. I feel almost confident that they are not. I feel strong doubts even whether the lines of group  $\alpha$ , though they give such a close approximation to simple Mendelian expectation, are in fact behaving in a simple Mendelian way. Such doubts are suggested by the fact that of lines 3 and 4, which are known to have a common ancestor in Crop C (cf. p. 304), line 3 has been placed in group  $\beta$  and line 4 in group  $\alpha$ . Lines 12 and 14 with a common ancestor in the same crop fall both into group  $\gamma$ , but lines 13, 15 and 16 which have a common ancestry fall, the first into group  $\gamma$ , and the second and third into group  $\alpha$ . The results suggest some sort of "splitting." Further, an examination of the frequency distributions of the percentages of each type of seed on the individual plants within each line showed some odd results. With only 20 or 30 dihybrid plants to a line distributions are necessarily very irregular, but the distributions for the percentages of YR seeds on plants of lines 6 (group  $\alpha$ ) and 24 (group  $\beta$ ) were so striking that they caught the eye at once. The distributions are given, with some others of somewhat

similar character in Table XX. It must be remembered that these distributions include plants of all sizes, however small the numbers of their seeds, and not those plants only with 100 seeds or more as in the work of Section IV.

It will be seen that in line 6 not a single plant shows a percentage of YR seeds between 52 and 56 inclusive—a range just below the Mendelian mode where frequency should be almost at its densest. Similarly, line 24 shows not a single plant with a percentage of YR seeds between 53 and 56 inclusive. Taking the frequency distribution for the totality of

TABLE XX.

*Showing the numbers of A plants with each percentage of YR seeds in five lines with remarkable distributions.*

Percentage of YR seeds on the plant	Number of plants					
	Line <i>M</i>					
	6	7	8	24	25	Total
38	—	—	—	1	—	1
39	—	—	—	—	—	—
40	—	—	—	—	—	—
41	—	—	—	—	—	—
42	—	—	1	—	—	1
43	—	—	1	—	—	1
44	1	1	—	—	—	2
45	—	—	—	—	—	—
46	—	—	—	—	1	1
47	—	—	1	—	—	1
48	—	—	—	2	1	3
49	2	—	1	1	—	4
50	1	—	1	3	2	7
51	3	—	2	1	1	7
52	—	3	3	2	2	10
53	—	3	1	—	—	4
54	—	—	1	—	—	1
55	—	1	—	—	—	1
56	—	—	—	—	2	2
57	1	1	1	2	2	7
58	3	4	—	4	2	13
59	—	1	4	5	—	10
60	2	—	2	—	5	9
61	—	2	—	1	2	5
62	1	2	2	7	2	14
63	1	2	2	1	1	7
64	—	2	—	1	1	4
65	—	1	—	—	—	1
66	1	1	—	—	—	2
67	1	—	—	—	—	1
68	—	—	—	—	—	—
69	—	—	—	—	—	—
70	—	—	—	—	—	—
71	—	—	—	—	—	—
72	—	—	—	—	—	—
73	1	—	—	—	—	1
Total	18	24	23	31	24	120



the lines, I find that the chance that a random selection of 18 plants should give no plant with a percentage between 52 and 56 as in line 6 is only .0030 or under 1 in 300; the chance that a random selection of 31 plants should give no plant with a percentage between 53 and 56 as in line 24 is only .00035 or under 1 in 2800. Lines 7, 8 and 25 seemed to show a somewhat similar result, with one or no observations over three intervals in a range close below the mode. The chance that only one observation should fall in the range 54 to 56 in a random selection of 24 plants as in line 7 is .055. The chance that only one observation should fall in the same range out of a random selection of 23 plants as in line 8 is .064, or that no observations should fall in the range 55-56 .047. The chance that no observations should fall in the range 53 to 55 out of a random selection of 24 plants as in line 25 is .018. Lines 7, 8 and 25 do not therefore give nearly such improbable results as lines 6 and 24, but they do give an unlikely divergence and show a curious similarity with the latter. Further, line 24 shows an odd peak at 62 per cent. which is emphasised in the aggregate of these five distributions. When working on the borders, or well within the limits, of fluctuations of sampling, it is very difficult to avoid picking up from a first inspection ideas as to exceptions or regularities which on further work prove to be apparently baseless, but so far as they go the distributions of these lines are again suggestive of the apparent compoundedness discussed in Section IV. The lower peak falls at 52, or at about 50-52; it falls at 52 in the distribution of the plants with 200 seeds or more given in Tables X and XI and illustrated in Fig. 7, and was tentatively placed at 51.5 in the dissection of the distribution for plants with 100 seeds or more. The peak at 58 or 58-59 also corresponds approximately with the maximum of the upper component both in the plants with 200 seeds or more and in the dissection of the distribution for plants with 100 seeds or more. But neither of the distributions of Section IV shows a peak at 62, which appears to arise from the smaller plants only. The distributions for the YW, GR and GW seeds of the dihybrid plants on these lines are very erratic, but do not give so marked a suggestion of compoundedness. It will be noted that of these five lines, which give such abnormal looking distributions for the percentages of YR seeds, lines 6 and 7 were assigned to group  $\alpha$  (the "approximately normal Mendelian" group), and lines 8, 24 and 25 to group  $\beta$ . Line 7 was rather abnormal in the proportions of its seeds, but line 6 was quite in fair agreement for Mendelian expectation both for seeds and for plants, yet it seems difficult to believe, with the distribution of Table XX in front of one, that it is really behaving in the way predicated by the simple Mendelian mechanism.

Finally, we may ask a question respecting these dihybrid plants which has not yet been dealt with. If we take out the percentages of round seeds, or yellow seeds—that is to say, of the single characters instead of the combinations—do these also suggest heterogeneity?

The data for the several lines are given in Table XXI, the proportions of rounds and of yellows per 10,000 of the total seeds in each line being taken by addition from Table XVI, a process which, it may be noted, may render the last digit inaccurate to a unit. After each proportion are given the two measures for the probability of occurrence on random sampling,  $P_1$  and  $P_2$ .  $P_1$  is the probability of the observed deviation from Mendelian expectation ( $\pm 500$ ), or a greater deviation of either sign, occurring on random sampling, and is obtained by taking the ratio of the observed deviation to the standard error (based on the Mendelian proportions  $\cdot 75$ ,  $\cdot 25$ ) and turning up the answer in the table of areas of the normal curve. As the chances are not equal, variation is not strictly normal, but with the numbers of observations here available the approximation must be close.  $P_2$  was calculated by a method that renders it comparable with the second  $P$  of the preceding tables. If  $p$ ,  $q$  are the proportions of the character in the total seeds of all the lines together, e.g. 0.7524, 0.2476 for the round seeds of Table XXI, the standard error of the difference between the proportions of rounds in any line and the aggregate of the other lines is

$$10000 \sqrt{pq} \sqrt{\frac{1}{n_1} + \frac{1}{n_2}},$$

where  $n_1$  is the number of seeds in the line and  $n_2$  the number in the aggregate of the remaining lines. The difference is taken between the proportion of seeds in the given line and the proportion in the aggregate of the remaining lines, this is divided by the above standard error, and the ratio is turned up in the tables of areas of the normal curve as before.

Considering the round seeds first, we see that the mean proportion is 7524 per 10,000, a deviation of 24 from Mendelian expectation; the standard error is 17.1, or the ratio of deviation to standard error 1.40. The chance of such a deviation, of either sign, occurring on random sampling is 0.16, or about 1 in 6. The average is hardly suggestive of anything abnormal. But the mean of  $P_1$  works out at only 0.3758, whereas on random sampling it should be 0.5. The standard error of the mean of  $P$ , as before, is 0.0536, and the ratio of 0.5–0.3758 to the standard error is 2.32, so that the probability of such a deviation in defect is only 0.01. The result is not so striking as the similar figures given above for the combinations of characters, but we feel almost bound to regard it as significant. Again, the mean value of  $P_2$  is only 0.3825, a deviation

of 0.1175 from expectation or 2.19 times the standard error; the probability of such a deviation in defect occurring on random sampling is only 0.014—say, about 1 in 70. While not rendering the result absolutely certain, these figures are at least fairly strong evidence that even as regards the distribution of the single pair of characters, roundness and wrinkledness, the separate lines of the dihybrid plants are not Mendelian and not a homogeneous group. The values of  $P$  will be found grouped by intervals of  $5/29$  (as in Table XIX) in Table XXIV, but this adds little to the weight of the conclusion. For  $P_1$ , the distribution is not very improbable; such a divergence from expectation might occur on almost

TABLE XXI.

*Showing for the A plants grouped by lines the proportion per 10,000 of round seeds in each line, the probability  $P_1$  of the observed deviation from Mendelian expectation on random sampling and the probability  $P_2$  of the observed deviation between the proportions given by each line and by the remainder; with similar information for the yellow seeds. The numbers of seeds will be found in Table XVI from which the proportions are taken by addition.*

Line: Ascendants of $M$	Round seeds per 10,000	$P_1$	$P_2$	Yellow seeds per 10,000	$P_1$	$P_2$
1	7361	.1835	.1118	7808	.0032	.0124
3	7495	.9601	.7490	7557	.5287	.9601
4	7362	.1211	.0615	7392	.2263	.0643
5	7396	.4777	.3785	7477	.8729	.6031
6	7352	.1556	.0930	7642	.1738	.3789
7	7681	.0404	.0688	7697	.0251	.0930
8	7333	.0561	.0251	7435	.4593	.1676
9	7395	.2585	.1556	7386	.2187	.0658
11	7423	.3320	.1868	7449	.5157	.1738
12	7722	.0183	.0324	7684	.0512	.1527
13	7651	.1645	.2380	7492	.9442	.5687
14	7678	.1389	.1936	7956	.0002	.0006
15	7383	.1770	.0949	7586	.3222	.6892
16	7558	.5093	.6965	7574	.4009	.8026
17	7512	.8966	.8887	7593	.2983	.6384
18	7491	.9283	.7339	7649	.1336	.3222
19	7613	.2891	.4009	7448	.6249	.3222
20	7546	.6241	.8181	7508	.9283	.6312
21	7549	.6672	.8259	7556	.6171	.9761
22	7620	.1738	.2713	7620	.1738	.4354
23	7564	.5157	.6818	7375	.2005	.0643
24	7484	.8181	.5419	7635	.0477	.2077
25	7544	.5823	.7949	7571	.3735	.8181
26	7596	.3472	.4777	7658	.1236	.2937
27	7682	.0160	.0316	7445	.4654	.1416
28	7520	.7642	.9442	7483	.8026	.2846
29	7833	.0629	.0819	7525	.8887	.8729
30	7441	.5287	.3681	7545	.6312	.9362
31	7652	.0910	.1471	7497	.9761	.5222
Weighted mean	7524	—	—	7553	—	—
Unweighted mean	7532	.3758	.3825	7560	.4148	.4207

one trial in five on random sampling, but it must be noticed that it is the two classes for low values of  $P$  that are in excess, containing 16 values of  $P$  against the expectation of 10, while all the others are in defect. For  $P_2$  the distribution is more irregular and  $P$  for the agreement with expectation is only 0.061; there are 15 observations in the two lowest classes against the expected 10.

For the proportions of yellows to greens the evidence of heterogeneity is in some respects much stronger, in others weaker. There are two lines which are almost certainly non-Mendelian. For line 1  $P_1$  is only about 1 in 300; for line 14 (·00015 to another place of decimals) about 1 in 7000. For this same line  $P_2$  is only about 1 in 1600. The presence of such lines almost precludes the series from being considered as Mendelian or as homogeneous. But the mean values of  $P$  are not so low as in the previous

TABLE XXII.

*Showing for B plants grouped by lines the proportion per 1000 of round seeds in each line and the probability  $P_1$  of the observed deviation from Mendelian expectation on random sampling.*

Line: Ascendants of $M$	Numbers of seeds			$R$ 's per 1000	$P_1$
	YR	YW	Total		
1	328	99	427	768	·3897
3	751	221	972	773	·0969
4	385	122	507	759	·6383
5	469	150	619	757	·6891
6	942	308	1250	754	·7414
7	818	274	1092	749	·9362
8	563	206	769	732	·2501
9	803	253	1056	760	·4533
11	1028	346	1374	748	·8650
12	1003	333	1336	751	·9362
13	715	244	959	746	·7718
14	648	240	888	730	·1676
15	787	281	1068	737	·3271
16	629	236	865	727	·1188
17	1051	349	1400	751	·9283
18	1316	442	1758	749	·9203
19	498	172	670	743	·6745
20	884	262	1146	771	·1010
21	871	298	1169	745	·6965
22	966	335	1301	743	·5619
23	873	252	1125	776	·0444
24	827	284	1111	744	·6455
25	806	292	1098	734	·2225
26	1012	330	1342	754	·7339
27	616	206	822	749	·9442
28	681	227	908	750	1·0000
29	377	141	518	728	·2460
30	1004	298	1302	771	·0801
31	1209	412	1621	746	·7114
Total	22860	7613	30473	750	—
Unweighted mean	—	—	—	750	·5480

case; the first differs from expectation by 1.59 times the standard error, the second by 1.48 times the standard error. The probabilities of these deviations in defect occurring in random sampling are .056 and .069. Table XXIV again adds little to the evidence.

Considering the data as a whole, there is evidence, which to me at least seems fairly convincing, that as regards the distribution of the single characters also the dihybrid lines are non-Mendelian in their behaviour and heterogeneous. but the divergences are not so marked nor striking as for the distribution of the two characters together.

The investigation is completed by Tables XXII and XXIII, on the same lines as Table XXI, for the monohybrid plants in each separate line of dihybrid descent. As the numbers of seeds in these tables are

TABLE XXIII.

*Showing for C plants grouped by lines the proportion per 1000 of yellow seeds in each line, and (1) the probability  $P_1$  of the observed deviation from Mendelian expectation on random sampling, (2) the probability  $P_2$  of the observed deviation between the proportions given by each line and by the remainder.*

Line: Ascendants of M	Numbers of seeds			Y's per 1000	$P_1$	$P_2$
	YR	GR	Total			
1	424	99	523	811	.0013	.0007
3	523	163	686	762	.4654	.3681
4	759	254	1013	749	.9442	.8887
5	424	134	558	760	.5823	.4839
6	1260	401	1661	759	.3953	.2340
7	535	185	720	743	.6672	.8103
8	702	238	940	747	.8337	1.0000
9	1103	367	1470	750	1.0000	.7949
11	1226	432	1658	739	.3030	.4654
12	598	226	824	726	.1118	.1499
13	563	215	778	724	.0930	.1285
14	425	130	555	766	.3843	.3077
15	907	308	1215	747	.8103	1.0000
16	961	284	1245	772	.0735	.0385
17	827	275	1102	750	1.0000	.8181
18	506	155	661	766	.3421	.2670
19	987	361	1348	732	.1260	.1868
20	797	283	1080	738	.3688	.5029
21	746	252	998	747	.8259	1.0000
22	828	270	1098	754	.7566	.5961
23	791	282	1073	737	.3271	.4593
24	828	313	1141	726	.0615	.0930
25	1306	461	1767	739	.2846	.4533
26	806	280	1086	742	.5419	.7114
27	949	305	1254	757	.5687	.4237
28	1554	539	2093	742	.3953	.6101
29	452	167	619	730	.2501	.3371
30	1471	476	1947	756	.5419	.3221
31	581	219	800	726	.1165	.1556
Total	23839	8074	31913	747	—	—
Unweighted mean	—	—	—	748	.4542	.4692

smaller and the average little exceeds 1000 per line, the proportions have only been worked out to the nearest unit per 1000 instead of per 10,000. In Table XXII the proportion of rounds in the aggregate of seeds for all the lines together agrees with Mendelian expectation to the nearest unit, and consequently there is no need for a second column for  $P_2$ . The mean value of  $P$  in this table actually comes out at 0.5480, in excess of expectation by .0480 or .90 times the standard error. The lines are in fact a closer fit to Mendelian expectation than might be expected on random sampling, though the fluctuation is quite within possible limits. The deviation for only a single line (line 23) barely exceeds twice the standard error. Of these deviations expressed in terms of the standard error 16 lie within the limits  $\pm 0.5$ , and 13 outside; there should be 11 inside and 18 outside. If the square of their standard deviation is worked out it comes to 0.8646 only instead of a unit. The result again raises the question whether the standard deviation of sampling for a group that is strictly "simple Mendelian" should be taken as less than the binomial value.

In the case of the  $C$  plants (Table XXIII) the mean proportion of yellows differs from expectation by 3.0 units per thousand or 1.34 times the standard error. The mean values of  $P_1$  and  $P_2$  are both lower than 0.5, but only by 0.85 times and 0.57 times the standard error. The distributions of  $P$  over the range 0 to 1, in Table XXIV (1), are very little suggestive of anything abnormal. There is one piece of evidence, and one only, which suggests heterogeneity in these plants also, and it seems to me to outweigh the rest. That evidence is line 1. This line gives 81.1 per cent. of yellow seeds on a total of 523. The chance of getting such a deviation in a simple Mendelian population is only 13 in 10,000; in a homogeneous population with an expectation of 74.7 per cent. of yellow seeds only

TABLE XXIV (1).

Showing the numbers of lines with values of  $P$  falling within certain limits, in Tables XXI to XXIII, together with the values of  $\chi^2$  and of  $P$  for the agreement of these figures with expectation on random sampling.

Value of $P$	Expectation	Number of lines, out of 29, for which $P$ falls within the limits on the left							
		A's, R:W $P_1$	A's, R:W $P_2$	A's, Y:G $P_1$	A's, Y:G $P_2$	B's $P_1$	C's $P_1$	C's $P_2$	
0-5/29	5	10	11	7	9	6	7	6	
5/29-10/29	5	6	4	7	6	4	5	6	
10/29-15/29	5	4	4	5	2	2	5	7	
15/29-20/29	5	4	2	4	6	5	5	2	
20/29-25/29	5	2	6	1	2	5	4	4	
25/29-1	4	3	2	5	4	7	3	4	
$\chi^2_P$	—	7.65 .19	10.60 .061	5.25 .39	7.20 .21	4.45 .49	1.25 .93	3.40 .64	

7 in 10000. The line contains eight *C* plants only, four of which give percentages of 82 or more. There is only one plant with over 100 seeds, *K* 1.9 with 134 seeds of which 114 or 85.1 per cent. are yellow; the deviation from Mendelian expectation is 2.70 times the standard error for this plant, and the chance of getting such a deviation of either sign on random sampling is 0.007. It seems to me practically impossible to regard such a line as Mendelian or as consistent with the homogeneity of the group.

I hoped that the differentiation noted between the lines of descent might throw some light on the remarkable variation in the proportions of seeds on the dihybrid plants from year to year which is brought out by Table VI (A). It seemed to me possible that the proportions of seeds contributed by the  $\beta$  and  $\gamma$  groups of lines might vary from crop to crop and so contribute to the fluctuation. I therefore took out the percentages of seeds on plants in the  $\beta$  and  $\gamma$  groups of lines to the seeds in all the 29 lines (not the total seeds in the crop) and obtained the following figures:

Crop	Percentage of seeds in $\beta$ and $\gamma$ groups of lines to seeds in all 29 lines
<i>G</i>	35.5
<i>H</i>	42.4
<i>J</i>	36.1
<i>K</i>	42.9
<i>L</i>	48.1
<i>M</i>	43.7

I can see no relation between the fluctuations exhibited by these percentages and the variation in the divergences from simple Mendelian expectation in Table VI (A). As a further test I took out separately the seeds on plants of the  $\alpha$ ,  $\beta$  and  $\gamma$  groups in Crops *K* and *M*, the first a crop giving very good agreement with Mendelian expectation in the totality of the seeds, the second the most divergent crop of the six. The

TABLE XXIV (2).

*Showing the numbers of the four types of seed on A plants in Crops K and M, together with the proportions per 10,000, distinguishing the three groups of lines  $\alpha$ ,  $\beta$  and  $\gamma$ .*

Crop	Group	Numbers of seeds					Proportions per 10,000			
		YR	YW	GR	GW	Total	YR	YW	GR	GW
<i>K</i>	$\alpha$	5172	1703	1736	632	9243	5596	1842	1878	684
	$\beta$	2379	816	797	239	4231	5623	1929	1884	565
	$\gamma$	1557	486	504	174	2721	5722	1786	1852	639
<i>M</i>	$\alpha$	3989	1390	1293	369	7041	5665	1974	1836	524
	$\beta$	1671	670	570	136	3047	5484	2199	1871	446
	$\gamma$	1431	468	433	93	2425	5901	1930	1786	384



results are shown in Table XXIV (2). It is clear that the causes which lead to variation in the proportions of seeds classified under the four heads from year to year act on plants of all three groups. In Crop *K*, the  $\alpha$  group shows a large excess of GW seeds, the  $\beta$  group still shows a deficiency, but the  $\gamma$  group gives a slight excess. In Crop *M*, all three groups show a heavy deficiency of GW's, but the deficiency is heaviest for the  $\beta$  and  $\gamma$  groups. In both crops, as in the total, the  $\beta$  and  $\gamma$  groups give the lowest percentages of GW's, and the  $\gamma$  group the highest percentage of YR's. The problem as to the source of variation in the proportions of seeds from year to year thus remains unsolved.

### *Summary.*

Summarising briefly the work of this section, we began by showing that far too many plants with abnormal proportions are found amongst the dihybrid plants (Table XIII). Some of the families in which the abnormal plants occurred proved to be very improbable aggregates (Table XIV) and there were more families than one would expect with two or three marked plants.

The last result suggested an investigation by lines of descent. The investigation showed that the different lines of dihybrid plants did not form a homogeneous group but differed significantly from each other; the conclusion being reached with almost complete confidence when the proportions of the four types of seed were considered, but with less confidence when the two characters (yellowness or roundness) were taken singly.

The deviations from Mendelian expectation noted for the totalities of the seeds and of the plants (Tables I and VI (A)) appear to be mainly due to about half the lines, the remainder giving totals not far from expectation.

For the monohybrid plants descended in the different dihybrid lines there was very much less evidence of heterogeneity. The hybrids for shape gave no evidence of heterogeneity at all. The hybrids for colour gave one line that was almost certainly abnormal.

It appears that the abnormalities which occur mainly (most largely or most frequently) affect the frequencies of combination of two characters, though they may also affect the distribution of the single character. Whatever the conditioning cause may be it appears to be something heritable, or the lines would not differentiate.

A line is not necessarily homogeneous (in the sense that a homozygous line is homogeneous) for two lines that are known to have descended from a common ancestor may differ significantly. It has even



been questioned whether a line that appears, from the proportions of different types of seeds and of plants, to be simply Mendelian, is in fact behaving according to the simple Mendelian rules, for the frequency distributions of percentages of YR seeds on single plants may be very abnormal. The result should be compared with the work of the last section on the large plants of Crops *J* and *K* (Tables XI, XII, and Fig. 7).

These results seem to me to enforce the tentative conclusion of the last section that the very simple mechanism usually postulated is inadequate to explain all the facts. It is not possible on such an assumption to account for separate lines of descent differing significantly in the proportions of seeds that they give, or in the proportions of YR seeds on dihybrid plants that prove to be dihybrid, monohybrid for shape, monohybrid for colour, and pure dominant.

#### VI. THE COMBINATIONS OF THE DIFFERENT TYPES OF SEED IN THE PODS.

In the previous sections we have considered first the agreement with expectation of the number of seeds of each type in the aggregate yielded by each generation, second, the agreement with expectation of the numbers of seeds of each type yielded by the individual plants. We now come to the further question, how far do the numbers of the four types of seed (YR, YW, GR, GW) in each pod, agree with Mendelian expectation—i.e. do they, or do they not, show nothing more than the fluctuations to be expected in random sampling?

It was decided to proceed in this case by way of sample, i.e. to investigate one generation only and to be guided by the results in deciding whether to carry the enquiry further or no, as a complete investigation would be very lengthy. Crop *G* ( $F_{12}$ ) seemed suitable enough for the preliminary trial. The seeds harvested on the *A* plants gave total numbers of the four types not diverging largely from expectation (cf. Table VI (A)), the probability of occurrence of the divergence observed, on random sampling, being about 0.05; it is convenient that the totals should show at least a moderate agreement with theory, for, primarily at least, we wish to know whether even on normal plants the pods may be regarded as nothing more than small random samples. I regret, in fact, that Crop *J* or Crop *K* was not chosen rather than *G* as they both give much closer agreement with Mendelian expectation. A supplementary investigation on one point was therefore made on these crops (Table XXVIII).

The pods of the *A* plants in Crop *G* were re-tabulated according to

the numbers of seeds they contained, omitting pods with one seed and pods with eight seeds or more—a relatively small proportion of the total. Table XXV shows the numbers of the four types of seed for each size of pod separately, together with the expected distributions in the ratio 9 : 3 : 3 : 1. It will be seen that most of the distributions, judging by the value of  $P$ , give very good agreement with expectation, the worst fit being given by the 6-seeded pods. In this case  $P$  is 0.014, so that roundly we might expect a fit as bad or worse on random sampling once in some 70 trials; as for the total seeds (of the  $A$  plants) yielded by this generation, the misfit is mainly due to a deficiency of YW's and GW's. The excesses or deficiencies as compared with expectation show singularly little agreement in the seeds from pods of different sizes, the signs of disagreement running as follows:

	Seeds in pod					
	2	3	4	5	6	7
YR	+	+	-	-	+	-
YW	-	-	+	-	-	+
GR	-	+	+	+	+	-
GW	-	-	-	+	-	+

No two distributions except those for the 3- and 6-seeded pods are alike in the signs of divergence, and the result given by the general aggregate is of the nature of an average of different distributions that vary considerably from each other. It may be pointed out that Table XXV gives a total of 5779 seeds against the general aggregate of 6017, so that no more than 238 seeds have been omitted by the exclusion of pods with fewer than two or more than seven seeds, and possibly the inadvertent omission of one or two pods within the range stated.

TABLE XXV.

*Numbers of seeds of each type in pods on dihybrid plants with 2, 3, 4, etc. to 7 seeds, Crop G, and probability P of the observed divergence from Mendelian expectation on random sampling.*

Seeds	Number of seeds in the pod											
	2		3		4		5		6		7	
	Obs.	The.	Obs.	The.	Obs.	The.	Obs.	The.	Obs.	The.	Obs.	The.
YR	83	76.5	272	266.6	595	605.25	969	973.1	928	884.25	437	444.9
YW	20	25.5	83	88.9	204	201.75	296	324.4	266	294.75	153	148.3
GR	25	25.5	93	88.9	212	201.75	349	324.4	303	294.75	144	148.3
GW	8	8.5	26	29.6	65	67.25	116	108.1	75	98.25	57	49.4
Total seeds	136	—	474	—	1076	—	1730	—	1572	—	791	—
Total pods	68	—	158	—	269	—	346	—	262	—	113	—
$P$	0.62	—	0.77	—	0.84	—	0.18	—	0.014	—	0.67	—

When Table XXV had been completed, the sheets for each size of pod were taken separately and tables compiled showing how many out of these pods contained 0, 1, 2, ... YR's; 0, 1, 2, ... YW's, and so on. The results are shown in Table XXVI (A) to (F). Thus, of the 68 2-seeded pods eight only contained no YR's, 37 contained one YR, and 23 contained two YR's. But 49 of them contained no YW, 18 only one YW, and only one two YW's. The other tables are read similarly.

Against each observed distribution, in the column headed "Obs.," is given the expected distribution, in the column headed "The." (Theory). If no definite causes are at work, and the fluctuations observed are due solely to the chances of random sampling, the numbers of pods with 0, 1, 2, ... seeds of the type considered should be given by the successive terms of the binomial series

$$N (q + p)^k,$$

where  $N$  is the number of pods for which data are available,  $k$  is the number of seeds in the pod,  $p$  is the chance of obtaining a seed of the kind considered (i.e. the proportion in which it should occur on Mendelian theory) and  $q$  is  $1 - p$ . Thus, for YR's  $q$  is 7/16 and  $p$  9/16; for YW's or GR's  $q$  is 13/16 and  $p$  3/16, and for GW's  $q$  is 15/16 and  $p$  1/16. It is these binomial distributions which are given in the columns headed "The." In most cases it will be seen that the consonance between theory and observation is extraordinarily good; the worst fits are shown by the distributions of YW's and GR's in the 4-seeded pods, where a fit as bad or worse would only be expected on random sampling once in 100 trials. This is a rather unexpected result and a warning that agreement of the average need not imply agreement of the distribution as a whole; for the numbers of YW and GR seeds in these pods, viz. 204 and 212 respectively (cf. Table XXV), are only slightly in excess of the expected number 202; the divergence that renders the distributions so improbable is mainly a marked excess of pods with three or four seeds of the type named, there being ten of such pods in the first case and 14 in the second against an expectation of only six. The expectation of pods with all of their four seeds YW's or GR's is very small, about one-third of a pod in a total of 269, but there are actually three of such pods in the distribution of YW's. The distribution of GR's in the 5-seeded pods shows a similar excess of pods with three seeds of that type, 25 against 15, but the excess is not general, and not necessarily significant, though consistent with the general excess of GR's; but the GW's are, on the whole, in defect.

We can get a general view of the consonance between theory and

TABLE XXVI.

Showing the frequency distributions of pods with given numbers of seeds of each type, in pods with 2-7 seeds, Crop G, and the probability *P* of the observed divergence from Mendelian expectation on random sampling<sup>1</sup>.

## (A) Pods with 2 seeds: Total, 68 pods

No. of seeds of type stated	Type of seed						
	YR		YW	GR		GW	
	Obs.	The.	Obs.	Obs.	The.	Obs.	The.
0	8	13	49	45	45	60	60
1	37	33	18	21	21	8	8
2	23	22	1	2	2	—	—
<i>P</i>	0.30	—	0.65	1.0	—	1.0	—

## (B) Pods with 3 seeds: Total, 158 pods

0	9	13	87	84	85	134	122
1	51	51	59	56	59	22	33
2	73	66	12	17	13	2	3
3	25	28	—	1	1	—	—
<i>P</i>	0.52	—	0.77	0.71	—	0.084	—

## (C) Pods with 4 seeds: Total, 269 pods

0	16	10	111	118	117	208	208
1	45	50	125	104	108	57	55
2	100	98	23	33	38	4	6
3	82	84	7	14	6	—	—
4	26	27	3	—	—	—	—
<i>P</i>	0.38	—	0.009	0.010	—	0.71	—

## (D) Pods with 5 seeds: Total, 346 pods

0	4	5	139	110	123	244	250
1	42	36	135	152	141	89	84
2	90	92	57	57	65	12	11
3	114	118	13	25	15	1	1
4	75	76	2	2	2	—	—
5	21	19	—	—	—	—	—
<i>P</i>	0.89	—	0.47	0.043	—	0.89	—

## (E) Pods with 6 seeds: Total, 262 pods

0	1	2	92	75	76	196	178
1	9	14	96	92	104	57	71
2	42	46	55	75	60	9	12
3	73	78	16	19	19	—	1
4	80	75	3	1	3	—	—
5	46	39	—	—	—	—	—
6	11	8	—	—	—	—	—
<i>P</i>	0.35	—	0.30	0.17	—	0.088	—

## (F) Pods with 7 seeds: Total, 113 pods

0	2	—	24	31	26	66	72
1	1	3	43	39	43	37	34
2	10	12	34	29	30	10	7
3	28	26	8	9	11	—	—
4	41	33	3	5	3	—	—
5	21	26	—	—	—	—	—
6	7	11	1	—	—	—	—
7	3	2	—	—	—	—	—
<i>P</i>	0.50	—	0.77	0.55	—	0.36	—

<sup>1</sup> The values of *P* have been worked out as the distributions stand, with the following exceptions: (C) YW's, 3 and 4 grouped; (E) YR's, 0 and 1 grouped, GW's, 2 upwards grouped; (F) YR's, 0 and 1 grouped, YW's, 4 upwards grouped.

observation by bringing together the values of  $P$ , of which there are 24. If there were nothing at work but the chances of random sampling, the observed values of  $P$  should be uniformly distributed over the range 0 to 1. Breaking up the range into fractions of  $1/6$ , four values of  $P$  should fall into each fraction. The actual distribution is as follows:

Value of $P$	Number of $P$ 's falling into the range given	
	Observed	Theory
0-1/6	4	4
1/6-1/3	4	4
1/3-1/2	4	4
1/2-2/3	4	4
2/3-5/6	3	4
5/6-1	5	4

The agreement could scarcely be closer; the probability of obtaining a worse fit on random sampling is over 0.98. In the data of Table XXVI, barring one or two divergences of detail that are not exhibited consistently by pods of different sizes, it is difficult to see anything but a very good illustration of the theory of sampling.

I decided, however, to apply a further and very stringent test. It may be argued that, while Table XXVI indicates nothing significant in the fluctuations of numbers of seeds of any particular type from pod to pod, it is nevertheless possible that there may be significant differences as regards the *combinations* of seeds occurring, i.e. that a particular combination of seeds (for example, 3 YR, 2 YW, 2 GR, 0 GW in a 7-seeded pod) may tend to occur much more often, or much more rarely, than would be expected on the theory of random sampling. It is difficult to imagine any mechanism under which the frequencies of individual types of seed would be subject to the laws of random sampling and the frequencies of combinations would not—but still, the thing is possible.

In order to see whether or no combinations can be regarded as random, within the pod, it is necessary to tabulate—for some chosen number of seeds in the pod—every possible combination of YR's, YW's, GR's and GW's that can occur; to work out the probability of each and thence the number of instances to be expected amongst the pods observed, and to compare this with the actual number observed<sup>1</sup>.

As my test case I chose pods with five seeds, as being the most frequent in Crop *J*, 346 of such pods being available. There are 56 possible combinations<sup>2</sup> of the four types of seed in a 5-seeded pod, as

<sup>1</sup> The order of seeds in the pod was not recorded.

<sup>2</sup> With  $n$  seeds in the pod, the number of possible combinations (order in the pod being disregarded) is  $\frac{(n+1)(n+2)(n+3)}{1.2.3}$ .

set out in Table XXVII. These are listed first of all by the number of YR's. The order for any given number of YR's is best explained by an example, say 2 YR's. The list for 2 YR's starts at No. 11; the number of GW's can be ignored, as only the YW's and GR's affect the order. The order is:

YR	YW	GR		YR	YW	GR
2	0	0		2	1	0
2	0	1	and its equally probable reciprocal	2	2	0
2	0	2	" " "			

and so on, the combinations beginning with 2 YR's, 1 YW following next.

Against each combination is placed its calculated probability; in the next column is given the observed number of times that combination was found in the pods, and in the next again the average number of times it should be found, obtained by multiplying the probability by the total number of pods (346). A glance down the table shows how good is the agreement between observation and calculation; the most notable

TABLE XXVII.

*Showing every possible combination of the four types of seed in a pod of five seeds, the probabilities of occurrence, and the observed and expected numbers of pods with each combination in the 346 5-seeded pods of Crop G.*

Combination					Proba- bility	Pods		Combination					Proba- bility	Pods	
YR	YW	GR	GW	Obs.		The.	YR	YW	GR	GW	Obs.	The.			
1	5	—	—	—	056314	21	19.5	29	1	4	—	—	003476	2	1.2
2	4	—	—	1	031285	9	10.8	30	1	1	1	2	004635	1	1.6
3	4	—	1	—	093856	39	32.5	31	1	1	2	1	013905	4	4.8
4	4	1	—	—	093856	27	32.5	32	1	2	1	1	013905	5	4.8
5	3	—	—	2	006952	3	2.4	33	1	1	3	—	013905	10	4.8
6	3	—	1	1	041714	21	14.4	34	1	3	1	—	013905	3	4.8
7	3	1	—	1	041714	12	14.4	35	1	2	2	—	020857	8	7.2
8	3	—	2	—	062571	20	21.6	36	—	—	—	5	000001	—	.0
9	3	2	—	—	062571	13	21.6	37	—	—	1	4	000014	—	.0
10	3	1	1	—	125141	45	43.3	38	—	1	—	4	000014	—	.0
11	2	—	—	3	000772	—	.3	39	—	—	2	3	000086	—	.0
12	2	—	1	2	006952	4	2.4	40	—	2	—	3	000086	—	.0
13	2	1	—	2	006952	2	2.4	41	—	—	3	2	000257	—	.1
14	2	—	2	1	020857	6	7.2	42	—	3	—	2	000257	—	.1
15	2	2	—	1	020857	11	7.2	43	—	—	4	1	000386	—	.1
16	2	—	3	—	020857	10	7.2	44	—	4	—	1	000386	—	.1
17	2	3	—	—	020857	7	7.2	45	—	—	5	—	000232	—	.1
18	2	1	1	1	041714	14	14.4	46	—	5	—	—	000232	—	.1
19	2	1	2	—	062571	18	21.6	47	—	1	1	3	000172	—	.1
20	2	2	1	—	062571	18	21.6	48	—	1	2	2	000772	—	.3
21	1	—	—	4	000043	—	.0	49	—	2	1	2	000772	—	.3
22	1	—	1	3	000515	1	.2	50	—	1	3	1	001545	2	.5
23	1	1	—	3	000515	—	.2	51	—	3	1	1	001545	1	.5
24	1	—	2	2	002317	1	.8	52	—	1	4	—	001159	—	.4
25	1	2	—	2	002317	1	.8	53	—	4	1	—	001159	—	.4
26	1	—	3	1	004635	2	1.6	54	—	2	2	1	002317	—	.8
27	1	3	—	1	004635	2	1.6	55	—	2	3	—	002317	1	.8
28	1	—	4	—	003476	2	1.2	56	—	3	2	—	002317	—	.8

divergences seem to occur owing to the deficiency of YW's and excess of GR's, so that in a pair of reciprocal combinations like 3 and 4, 6 and 7, 8 and 9, which should be equally frequent, the first member of the pair usually shows an excessive (or less deficient) frequency, the latter a more deficient frequency.

To apply the  $\chi^2$  test of fit it is clearly necessary to group the frequencies in some way, since many of the expected frequencies are very small. Grouping together all the cases (35) in which the expected frequency is less than three units, which show a total of 25 pods against an expectation of 22.6, the value of  $\chi^2$  for the distribution thus condensed into 22 classes is 20.98 and of  $P$  0.46—indicating an exceedingly satisfactory agreement, notwithstanding the divergences noted due to the relative deficiency of YW's. If we, in part at least, eliminate this source of disturbance by pooling together each pair of reciprocals, thus reducing the total number of groups to 14,  $P$  rises to no less than 0.885, indicating a far closer agreement than we have any right often to expect.

Apart from the small amount of interchange between YW's and GR's, which is itself well within the limits of fluctuations of sampling, there is nothing, so far as I can see, even remotely to suggest anything significant in the relative frequencies with which different combinations of seeds occur in the pods.

Since, so far as I am aware, no similar analysis has ever been made of the relative frequencies of different combinations of seeds in a pod, the result seemed worth controlling by carrying out the test on another crop. It was decided to utilise both Crops *J* and *K*, which gave quite close agreement with Mendelian expectation in the totals of the seeds from dihybrid plants (cf. Table VI (A)). Table XXVIII gives the results, which, it will be seen, are based on much more extensive material than those of Table XXVII, 906 pods being available from Crop *J* and 854 from Crop *K*. For the same reduction to 22 classes as was used for Table XXVII,  $\chi^2$  for Crop *J* comes to 20.71, and  $P$  to 0.48. For the data of Crop *K*,  $\chi^2$  comes to 26.80 and  $P$  to 0.18. The fluctuations for both crops, taken as a whole, lie therefore well within the limits of fluctuations of sampling, but Crop *K* gives the poorer agreement with expectation. The divergence is largely due to combination 35 (1 YR, 2 YW, 2 GR, 0 GW) for which *K* gives 30 pods against an expectation of barely 18, and next to combination 34 (1 YR, 3 YW, 1 GR, 0 GW) for which *K* gives only five pods against an expectation of nearly 12.

When the numbers of pods for Crops *J* and *K* are added together,  $\chi^2$  comes to 22.01 and  $P$  to 0.40, the divergences in *J* and *K* partly cancelling out. It will be seen in fact that, for the great bulk of the



TABLE XXVIII.

Showing, as in Table XXVII, every possible combination of the four types of seed in pods with five seeds and the observed and expected numbers of pods with each combination, in the 906 5-seeded pods of Crop J, the 854 5-seeded pods of Crop K, and the 1760 5-seeded pods of the two crops together.

Combina- tion	J pods		K pods		J and K pods		Combina- tion	J pods		K pods		J and K pods		K pods		J and K pods	
	Obs.	The.	Obs.	The.	Obs.	The.		Obs.	The.	Obs.	The.	Obs.	The.	Obs.	The.	Obs.	The.
1	47	51.0	49	48.1	96	99.1	29	1	3.1	3	3.0	4	6.1	3	3.0	4	6.1
2	23	28.3	33	26.7	56	55.0	30	4	4.2	1	4.0	5	8.2	1	4.0	5	8.2
3	73	85.0	88	80.2	161	165.2	31	10	12.6	14	11.9	24	24.5	14	11.9	24	24.5
4	88	85.0	83	80.2	171	165.2	32	14	12.6	12	11.9	26	24.5	12	11.9	26	24.5
5	10	6.3	1	5.9	11	12.2	33	14	12.6	9	11.9	23	24.5	9	11.9	23	24.5
6	38	37.8	31	35.6	69	73.4	34	17	12.6	5	11.9	22	24.5	5	11.9	22	24.5
7	35	37.8	35	35.6	70	73.4	35	21	18.9	30	17.8	51	36.7	30	17.8	51	36.7
8	47	56.7	44	53.4	91	110.1	36	—	—	—	—	—	—	—	—	—	—
9	46	56.7	55	53.4	101	110.1	37	—	—	—	—	—	—	—	—	—	—
10	127	113.4	98	106.9	225	220.3	38	—	—	—	—	—	—	—	—	—	—
11	—	—	1	7	1	14	39	—	—	—	—	—	—	—	—	—	—
12	10	6.3	4	5.9	14	12.2	40	—	—	—	—	—	—	—	—	—	—
13	6	6.3	3	5.9	9	12.2	41	—	—	—	—	—	—	—	—	—	—
14	31	18.9	22	17.8	53	36.7	42	—	—	—	—	—	—	—	—	—	—
15	21	18.9	16	17.8	37	36.7	43	1	3	1	3	2	6	1	3	2	6
16	23	18.9	14	17.8	37	36.7	44	—	—	—	—	—	—	—	—	—	—
17	20	18.9	17	17.8	37	36.7	45	—	—	—	—	—	—	—	—	—	—
18	36	37.8	43	35.6	79	73.4	46	—	—	—	—	—	—	—	—	—	—
19	59	56.7	55	53.4	114	110.1	47	—	—	—	—	—	—	—	—	—	—
20	60	56.7	60	53.4	120	110.1	48	1	7	1	7	1	14	1	7	1	14
21	—	—	—	—	—	—	49	—	—	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	50	2	1.4	1	1.3	2	2.7	3	1.3	2	2.7
23	1	5	1	4	2	9	51	1	1.4	1	1.3	1	2.7	1	1.3	2	2.7
24	3	2.1	—	2.0	3	4.1	52	1	1.1	—	1.0	2	2.1	—	1.0	2	2.1
25	—	—	2	2.0	2	4.1	53	3	1.1	—	1.0	5	2.1	—	1.0	5	2.1
26	4	4.2	2	4.0	6	8.2	54	1	2.1	3	2.0	4	4.1	3	2.0	4	4.1
27	2	4.2	4	4.0	6	8.2	55	2	2.1	4	2.0	6	4.1	4	2.0	6	4.1
28	1	3.1	1	3.0	2	6.1	56	—	—	—	—	—	—	1	—	—	—

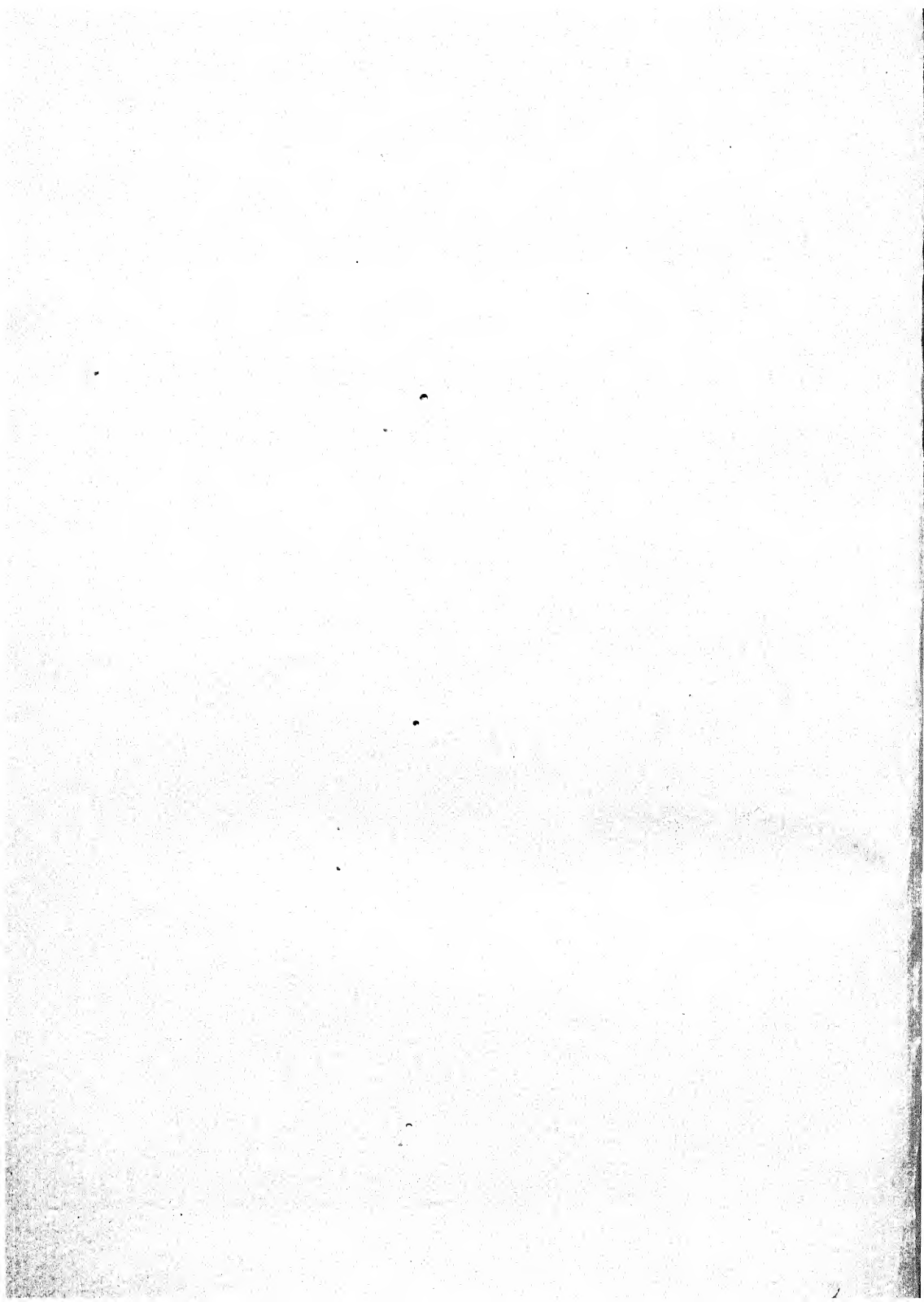


combinations, the agreement with expectation is very close; more than half the value of  $\chi^2$  is contributed by the two combinations 14 (2 YR, 0 YW, 2 GR, 1 GW) and 35 (1 YR, 2 YW, 2 GR, 0 GW). For combination 14 the two crops together give 53 pods against an expectation of barely 37, and for combination 35, 51 pods against the same expectation. In the case of combination 34, for which the number of pods in *K* is deficient, the number of pods in *J* is excessive and the agreement in the total quite close. For combination 14 the deviation is 2.72 times the standard error, the chance of occurrence of such a deviation (of either sign) on random sampling being 0.0065 or about 1 in 150 or 160. For combination 35 the deviation is 2.39 times the standard error for which the same chance is 0.017, or about 1 in 60. One such divergence as that for combination 35 in a series of 56 may well pass—it is indeed to be expected—but the divergence of 14 seems a little excessive; one can hardly say more.

No influence, therefore, is apparent of the divergences from simple Mendelian expectation noted in the preceding sections, and this is perhaps hardly to be expected. With only five seeds in the pod, the influence of pure chance would probably be overwhelming, and the slight divergences of the chances from Mendelian values would have little effect.

### *Summary.*

The work of the present section shows that the frequency distributions of numbers of seeds of each type in pods of one and the same size, and the frequencies of occurrence of the possible combinations, are not suggestive of any appreciable departure from the expectation given by random sampling out of a "population" of seeds in the proportions 9:3:3:1. Appreciable divergence owing to the abnormalities noted in previous sections was perhaps hardly to be expected, but the test seemed worth carrying out, as such a test does not appear to have been made before.



# THE INHERITANCE OF WING COLOUR AND PATTERN IN THE LEPIDOPTEROUS GENUS *TEPHROSIA* (*ECTROPIS*) WITH AN ACCOUNT OF THE ORIGIN OF A NEW ALLELOMORPH.

## I. EXPERIMENTS INVOLVING MELANIC *TEPHROSIA CREPUSCULARIA*.

By J. W. HESLOP HARRISON, D.Sc., F.R.S.E.

(With Plate XVIII.)

FOR several years I have been investigating the inheritance of melanism in the two Boarmiine genera *Tephrosia* and *Boarmia*. As a result of my researches I discovered that, without exception, whenever any one of the species *Tephrosia consonaria*<sup>1</sup>, *T. crepuscularia* and *Boarmia consortaria*<sup>1</sup> was crossed with its melanic variety the inheritance proved to be of the usual Mendelian type. But in the inter-specific crosses between *Tephrosia bistortata* and the melanic form of its congener, *T. crepuscularia*, the character in question behaved in quite an anomalous fashion<sup>2</sup>, and further experiments were consequently undertaken.

In this second series of cultures two races of *Tephrosia bistortata* were employed, (1) a double-brooded Kent strain, and (2) a single brooded race from Durham and the Cleveland District of Yorkshire. Delamere Forest, the well-known habitat of the form, provided the two *delamerensis* females with which the work commenced; of these females perhaps some description seems necessary here. In colour, the wings and body of the first were of a pure bluish-black, the former being traversed by the clear, narrow white subterminal line or rather band which characterises the black forms of so many Boarmiinae like *Boarmia repandata*, *B. consortaria*, *Tephrosia luridata* and others.

Her progeny resembled their mother very closely, thus demonstrating that at least one of their parents had been homozygous for

<sup>1</sup> I withheld my results in these species when I heard that Onslow was experimenting with them. It is sufficient to say at this juncture that my work harmonises with his.

<sup>2</sup> See *Journal of Genetics*, No. 1, 1920.

melanism. By inbreeding a promiscuous lot of this first captive generation, by crossing certain of its members with a Staffordshire batch known to be heterozygous for the same character, and by making the two possible crosses between it and type *Tephrosia bistortata*, it was easily shown that this stock, and therefore both its wild parents, had also been homozygous for colour.

The fact that the offspring of the second female (which was distinctly lighter in tone than the first) comprised both melanics and types indicated that it had been heterozygous for melanism.

#### BREEDING EXPERIMENTS.

Reciprocal pairings between *T. crepuscularia* var. *delamerensis* and both races of *bistortata* were made and the possible  $F_1$  lots brought to maturity with results, in respect to sex and colour, displayed in Tables I, II A and II B.

TABLE I.

*Homozygous type bistortata* ♀ × *homozygous melanic crepuscularia* ♂.  
*mm* × *MM*.

Family	Source of Female	Source of Male	Melanics			Types	Percentage Melanic
			Males	Females	Totals		
$D_1$	Kent	Cheshire	109	7	116	None	100
$D_2$	"	"	93	0	93	"	"
$D_3$	"	"	87	0	87	"	"
$D_4$	"	"	38	0	38	"	"
$D_5$	"	"	46	0	46	"	"
$e$	Eston, N. Yorks	"	6	25	31	"	"
$f$	"	"	21	29	50	"	"
$g$	Kildale, N. Yorks	"	30	41	71	"	"
$h$	N. Durham	"	7	5	12	"	"
Actually reared			...	...	544	0	100
Theoretical result			...	...	All	0	100

TABLE II A.

*Homozygous melanic crepuscularia* ♀ × *homozygous type bistortata* ♂.  
*MM* × *mm*.

Family	Source of Female	Source of Male	Melanics			Types	Percentage Melanic
			Males	Females	Totals		
$D_6$	Cheshire	Kent	54	58	112	None	100
$D_7$	"	"	52	60	112	"	"
$D_8$	"	"	60	67	127	"	"
$i$	"	North Durham	34	38	72	"	"
$j$	"	Eston	21	19	40	"	"
$k$	"	Kildale	27	28	55	"	"
Actually reared			...	...	518	0	100
Expected			...	...	All	0	100

TABLE II B.

*Heterozygous melanic crepuscularia* ♀ × *homozygous type bistortata* ♂.*Mm* × *mm*.

Family	Source of Female	Source of Male	Melanics			Types			Percentage Melanic
			Males	Females	Total	Males	Females	Total	
<i>D</i> <sub>9</sub>	Cheshire	Kent	14	18	32	10	18	28	53.3
Theoretical result			...	...	30	—	—	30	50

The data suggest that the melanic form behaves as a simple dominant to the type, but, in the light of the earlier work, this is no decisive proof, the condition of the *F*<sub>1</sub> lots being the "acid" test. Unfortunately, for various reasons, such as the precocious emergence of the females in crosses between *T. crepuscularia* females and the males of both stocks of *bistortata*, and the extraordinarily low viability of the females in the reverse cross between bivoltine *bistortata* females and *crepuscularia* males, the *F*<sub>2</sub> generations are not easily obtained. However, I managed to secure matings representative of all the necessary combinations, in addition to pairings between (univoltine *bistortata* ♀ × *crepuscularia* var. *delamerensis* ♂) females on the one hand, and the males of the two reciprocal *F*<sub>1</sub> hybrids involving double-brooded *bistortata* and *crepuscularia* var. *delamerensis* on the other.

Further, I obtained several back crosses, two of which were represented by a fairly large number of pairings, in addition to certain other instructive crosses.

From all of these matings eggs were secured, and the resultant broods, started on a diet of hawthorn (*Crataegus Oxyacantha*) and finished on knotgrass (*Polygonum aviculare*) were brought to the imaginal state; their composition in respect to the essential features, as well as that of other pertinent batches, may be determined from Tables III, IV A, IV B, V A, V B, V C, V D, V E and VI.

TABLE III.

*F*<sub>2</sub> broods reared from type *bistortata* ♀ × (*homozygous melanic*) *crepuscularia* ♂.*Mm* × *Mm*.

Family	Melanics			Types			Percentage Melanic
	Males	Females	Totals	Males	Females	Totals	
<i>F</i> <sub>2</sub> <i>D</i> <sub>1</sub>	1	7	8	3	3	6	—
<i>e</i> ♀ × <i>D</i> <sub>5</sub> ♂	34	30	64	11	12	23	—
Actually reared			72	—	—	29	71.9
Expectation			75.7	—	—	25.2	75

TABLE IV A.

*F*<sub>2</sub> broods bred from crepuscularia ♀ (homozygous melanic) × bistortata ♂ (type).*Mm* × *Mm*.

Family	Melanics			Streaks			Types		
	Males	Females	Totals	Males	Females	Totals	Males	Females	Totals
<i>F</i> <sub>2</sub> <i>D</i> <sub>8</sub>	15	17	32	5	6	11	8	6	14
<i>F</i> <sub>2</sub> <i>i</i>	4	5	9	—	—	—	4	1	5
<i>F</i> <sub>2</sub> <i>j</i>	6	12	18	3	1	4	3	4	7
<i>F</i> <sub>2</sub> <i>k</i>	2	5	7	—	—	—	2	1	3

Theoretical expectation in all of these broods is 3 melanic : 1 type.

TABLE IV B.

*F*<sub>2</sub> broods bred from crepuscularia ♀ (heterozygous melanic) × bistortata ♂ (homozygous type). *Mm* × *mm* or *mm* × *Mm*.

Family	Female	Male	Melanics			Types			Percentage Melanic
			Males	Females	Totals	Males	Females	Totals	
<i>F</i> <sub>2</sub> <i>D</i> <sub>0</sub>	Melanic	Type	23	17	40	16	16	32	55.5
<i>F</i> <sub>2</sub> <i>D</i> <sub>0</sub>	Type	Melanic	16	25	41	29	15	44	48.2
Actually reared			...	81	—	—	76	51.5	
Theoretical result			...	78.5	—	—	78.5	50	

TABLE V A.

Back crosses between bistortata ♀ ♀ and *F*<sub>1</sub> (bistortata ♀ × crepuscularia ♂) ♂ ♂.

Family	Type of Mating	Melanics			Types		
		Males	Females	Totals	Males	Females	Totals
bistortata ♀ × <i>D</i> <sub>3</sub> ♂	<i>mm</i> × <i>Mm</i>	24	13	37	32	15	47
„ × <i>D</i> <sub>2</sub> ♂	<i>mm</i> × <i>Mm</i>	41	22	63	55	30	85
„ × <i>D</i> <sub>2</sub> ♂	<i>mm</i> × <i>Mm</i>	32	19	51	57	22	79
„ × <i>D</i> <sub>3</sub> ♂	<i>mm</i> × <i>Mm</i>	21	9	30	24	14	38
10 bist. ♀ ♀ × 10 <i>D</i> <sub>5</sub> ♂ ♂	<i>mm</i> × <i>Mm</i>	55	26	81	57	33	90
Actually reared		...	262	—	—	339	
Expected		...	300.5	—	—	300.5	

TABLE V B.

Back crosses between bistortata ♀ ♀ and *F*<sub>1</sub> (crepuscularia ♀ × bistortata ♂) ♂ ♂.

Family	Type of Mating	Melanics			Types		
		Males	Females	Totals	Males	Females	Totals
bistortata ♀ × <i>D</i> <sub>6</sub> ♂	<i>mm</i> × <i>Mm</i>	27	16	43	28	12	40
„ × <i>D</i> <sub>6</sub> ♂	<i>mm</i> × <i>Mm</i>	30	18	48	35	18	53
10 bist. ♀ ♀ × 10 <i>D</i> <sub>6</sub> ♂ ♂	<i>mm</i> × <i>Mm</i>	52	25	77	45	21	66
Actually reared		...	168	—	—	159	
Expected		...	163.5	—	—	163.5	

TABLE V c.

Back crosses between  $F_1$  (crepuscularia ♀ × bistortata ♂) ♀ ♀ and bistortata ♂ ♂.

Family	Type of Mating	Melanics			Types			Actual Ratio	Expected Ratio
		Males	Females	Totals	Males	Females	Totals		
$D_8$ ♀ × bistortata ♂	$mm \times mm$	—	—	—	43	39	82	All types	All types
$D_8$ ♀ × „	$Mm \times mm$	33	28	61	29	26	55	1:1:1	1:1
$e$ ♀ × „	$Mm \times mm$	25	26	51	15	25	40	1:8	1:1

TABLE V d.

Back cross between  $F_1$  (crepuscularia ♀ × bistortata ♂) ♀ and crepuscularia ♂.

Family	Type of Mating	Melanics			Types			Actual Ratio	Expected Ratio
		Males	Females	Total	Males	Females	Total		
$D_8$ ♀ × crepuscularia ♂	$Mm \times MM$	41	28	69	—	—	—	All melanic	All melanic

TABLE V e.

Back crosses between  $F_1$  (crepuscularia ♀ × bistortata ♂) ♀ ♀ and bistortata ♂ ♂ which yielded anomalous results.

Family	Seeming Type of Mating	Melanics			Blotched			Type			Expected Ratio
		Males	Females	Totals	Males	Females	Totals	Males	Females	Totals	
$D_8$ ♀ × bistortata ♂	$Mm \times mm$	5	6	11	3	1	4	8	7	15	1 mel. : 1 type
$D_8$ ♀ × „	$Mm \times mm$	0	1	1	0	1	1	31	75	106	1 mel. : 1 type
$D_8$ ♀ × „	$mm \times mm$	31	25	56	—	—	—	8	11	19	All types

TABLE VI.

Crosses involving the reciprocal  $F_1$  broods.

Family	Type of Mating	Melanics			Types			Theoretical Ratio	Actual Ratio
		Males	Females	Totals	Males	Females	Totals		
$D_8$ ♀ × $D_8$ ♂	$Mm \times Mm$	30	13	43	10	5	15	3:1	2:86:1
$D_8$ ♀ × $W_1$ ♂	$Mm \times mm$	13	9	22	16	10	26	1:1	85:1
$e$ ♀ × $D_8$ ♂	$Mm \times Mm$	5	6	11	2	2	4	3:1	2:75:1

\*  $W_1$  was an  $F_1$  bistortata ♀ × type crepuscularia ♂ hybrid.

The outstanding points here are the advent of blotched or streaked insects, manifesting a more or less coarse mosaic of the type and melanic facies, in four of the families, and the failure of expectation, based on the appearance of the parent females, in the last two families of Table V e.

Unlike the condition of the  $F_2$  hybrids bred from the crossing of a *delamerensis* female with a *bistortata* male discussed in my earlier publication, family  $F_2 D_8$  of similar parentage displayed no great spread of variation. Instead the brood includes a selection of well-marked type insects, like the male grand-parent, with a greater proportion of

definitely melanic examples reproducing the condition of the other grand-parent *T. crepuscularia* var. *delamerensis*. In addition, however, to insects of these two forms, which were quite in accordance with one's expectations had the characters in question "mendelised" normally, there were the mosaics to which reference has just been made. These, like their black and their typical relatives, comprised individuals of each sex. Obviously, they replace a portion of the heterozygous melanics which ought to have formed one-half of the brood.

Regarding them as such, and adding them to the genuine melanics, we get a ratio of 43 melanics to 14 types (3.1 : 1), or practically the ordinary 3:1 monohybrid ratio. This would seem to suggest that insects of this phenotype might represent the heterozygous melanics. That such a conception is erroneous, however, is clear from several considerations.

In the first place, the heterozygotes in the  $F_1$  batch show no such anomalous wing colour, and, secondly, several of the  $F_2$  melanics, inseparable on a colour basis from the other members of the same group, when tested by the experiments listed in Table VII below, proved to be heterozygous for melanism. Probably, therefore, this new type is the outcome of interspecific hybridity, arising perhaps in some such manner as I suggested in my 1920 paper.

As mentioned previously family  $F_2 D_8$  was not the only batch exhibiting insects of this form, for specimens resembling them in every particular were reared in the  $F_2$  lot derived by pairing a *T. crepuscularia* var. *delamerensis* female with a univoltine *bistortata* male, as well as in the two back crosses between  $F_1$  (*crepuscularia* var. *delamerensis* ♀ × *bistortata* ♂) females and bivoltine *bistortata* males. In two of these instances the new variety occurred in each sex, whilst in the third one female alone was blotched.

In marked contrast to these cases, which arose from hybrid females of origin *delamerensis* ♀ × *bistortata* ♂, in no brood in which either sex of the reciprocal *bistortata* ♀ × *crepuscularia* var. *delamerensis* ♂ hybrid participated, whether the *bistortata* were single or doublebrooded, did any such mosaics appear.

#### THE INHERITANCE OF MELANISM.

The facts recorded in Tables I—VI show that, in the *T. bistortata* ♀ × *delamerensis* ♂ hybrids, melanism behaves as a simple unit-character although, perhaps, the persistent deficiency of the melanics in the backcrosses between pure *bistortata* females and males of such parentage



seems significant. Its importance, however, is discounted by the fact, discussed in my paper on the sex ratios in these hybrids, that one-half of the females in such back crosses are non-viable, coupled with the observation that, in most broods containing melanic insects, the melanic females outnumber the types of the same sex.

Nor does the evidence of the back crosses arising from (*delamerensis* ♀ × *bistortata* ♂) males, and of the *e* ♀ × *bistortata* ♂ batch, weaken the position. But what about the melanism in the family  $F_2$   $D_8$  containing the mosaics? Does the melanism there behave normally in future crosses or do more mosaics appear? To test this several matings were made between pure *T. bistortata* on the one hand, and true melanics and types from the abnormal  $F_2$  lot on the other. The result is shown in Table VII.

TABLE VII.

Family	Type of Mating	Melanics			Types			Expected Ratio	Actual Ratio
		Males	Females	Totals	Males	Females	Totals		
$F_2$ $D_8$ ♀ × <i>bist.</i> ♂	<i>MM</i> × <i>mm</i>	51	54	105	—	—	—	All melanics	All melanics
$F_2$ $D_8$ ♀ × <i>bist.</i> ♂	<i>Mm</i> × <i>mm</i>	14	15	29	16	16	32	1 : 1	1 : 1.1
$F_2$ $D_8$ ♀ × <i>bist.</i> ♂	<i>mm</i> × <i>mm</i>	—	—	—	47	43	90	All types	All types
<i>bist.</i> ♀ × $F_2$ $D_8$ ♂	<i>mm</i> × <i>Mm</i>	15	19	34	17	15	32	1 : 1	1 : .95

Everything here shows that melanism, once it has escaped drastic change during the gametogenesis of the  $F_1$  *crepuscularia* ♀ × *bistortata* ♂ hybrids, displays nothing unusual in its inheritance, although it is perfectly clear that some of the  $F_2$  insects employed had been heterozygous, and others homozygous, for melanism. Nevertheless, to clinch matters members of the brood set out first in Table VII were inbred, yielding as a result 87 insects, 66 melanics and 21 types, in other words, complete agreement with Mendelian anticipations.

To demonstrate further that melanism, in generations later than hybrids of the  $F_2$  order, as well as in extracted types<sup>1</sup> from various sources, presented no anomalies in heredity the following complex crosses, giving the figures in Table VIII, were made:

(m)  $bi^d*$  type ♀ × ( $bi^d$  ♀ ×  $D_8$  ♂) type ♂.

(n) ( $D_8$  ♀ ×  $D_8$  ♂) type ♀ ×  $F_1$  (*cr* ♀ ×  $bi^d$  ♂) ♂ (type).

(o) ( $bi^d$  ♀ ×  $D_8$  ♂) type ♀ × *cr* ♂.

(p) {type ♀ (*ex o*) ×  $F_1$  ( $bi^d$  ♀ × *cd* ♂) ♂} type ♀ × (*e* ♀ ×  $D_8$  ♂) type ♂.

\*  $bi^d$ =double-brooded *bistortata*;  $bi^s$ =single-brooded *bistortata*; *cr*=*crepuscularia* type; *cd*=*crepuscularia* var. *delamerensis*.

<sup>1</sup> Table XI should also be consulted.

- (q)  $bi^d \text{♀} \times (e \text{♀} \times D_s \text{♂}) \text{♂}$  (melanic).  
 (r)  $(D_s \text{♀} \times W_1 \text{♂})$  melanic  $\text{♀} \times (D_s \text{♀} \times W_1 \text{♂})$  type  $\text{♂}$ .  
 (s)  $(D_s \text{♀} \times D_s \text{♂})$  type  $\text{♀} \times F_1 (bi^d \text{♀} \times cd \text{♂})$  melanic  $\text{♂}$ .  
 (t)  $bi^d \text{♀} \times \{o \text{ type } \text{♀} \times F_1 (bi^d \text{♀} \times cd \text{♂}) \text{♂}\}$  type  $\text{♂}$ .  
 (u)  $e$  melanic  $\text{♀} \times (bi^d \text{♀} \times D_s \text{♂})$  type  $\text{♂}$ .  
 (v)  $(bi^d \text{♀} \times D_s \text{♂})$  type  $\text{♀} \times$  heterozygous  $cd \text{♂}$ .  
 (w)  $(bi^d \text{♀} \times D_s \text{♂})$  type  $\text{♀} \times bi^d \text{♂}$ .  
 (x)  $o \text{ type } \text{♀} \times F_1 (bi^d \text{♀} \times cd \text{♂})$  melanic  $\text{♂}$ .  
 (y)  $(e \text{♀} \times D_s \text{♂})$  melanic  $\text{♀} \times bi^d \text{♂}$ .

TABLE VIII.

Miscellaneous crosses showing the further behaviour of melanics and extracted types.

Family	Type of Mating	Melanics			Streaks			Types			Expected Ratio	Actual Ratio
		Males	Females	Totals	Males	Females	Totals	Males	Females	Totals		
m	$mm \times mm$	—	—	—	—	—	—	50	25	75	{ All types	All types
n	$mm \times mm$	—	—	—	—	—	—	27	24	51	{ All types	All types
o	$mm \times mm$	—	—	—	—	—	—	38	17	55	{ All types	All types
p	$mm \times mm$	—	—	—	—	—	—	29	26	55	{ All types	All types
q	$mm \times Mm$	19	9	28	—	—	—	16	7	23	1:1	1.2:1
r	$Mm \times mm$	8	12	20	—	—	—	9	7	16	1:1	1.2:1
s	$mm \times Mm$	10	5	15	—	—	—	12	5	17	1:1	.89:1
t	$mm \times mm$	—	—	—	—	—	—	29	19	48	{ All types	All types
u	$Mm \times mm$	18	17	35	—	—	—	16	13	29	1:1	1.2:1
v	$mm \times Mm$	11	7	18	—	—	—	12	8	20	1:1	1:1.1
w	$mm \times mm$	—	—	—	—	—	—	68	59	127	{ All types	All types
x	$mm \times Mm$	24	20	44	—	—	—	24	17	41	1:1	1:1
y	$Mm \times mm$	19	18	37	—	—	—	18	13	31	1:1	1.1:1

Once again, to judge from the table, melanism must be regarded as a Mendelian unit-character dominant to the type, acting quite typically in generations later than the  $F_2$  lots.

#### THE GENETICAL BEHAVIOUR OF THE MOSAICS.

In general, most of the examples of the new variety gave one the idea that they formed a very rough mosaic of the two main forms. In others, on the contrary, so fine and regular was the coloration, and so brown in tone the melanin to which the colour was due, that one could almost regard the moths as dilute blacks comparable with the dilute colours encountered in various other animals. This was the first indication that I was possibly dealing with the spontaneous

development of the third member of a series of multiple allelomorphs for colour.

To test the point, a nicely marked average female specimen was paired up with a second brood *T. bistortata* male, and, similarly, a mosaic female, with the division between the typical and the melanic portions of the wing and body areas sharply marked, was mated with a heterozygous  $F_1$  hybrid from  $D_2$ . The results, given in Table IX, lend complete support to the view that the new character "streak"<sup>1</sup> (mosaic) forms a simple allelomorph, dominant to the type and recessive to black, for we obtain nothing but such streaks and types in the first test brood, and melanics, streaks and types in the second.

TABLE IX.

Family	Type of Mating	Melanics			Streaks			Types			Expected Ratio	Actual Ratio
		Males	Females	Totals	Males	Females	Totals	Males	Females	Totals		
$F_2 D_8 \text{♀} \times \text{bist. ♂}$	$Sm \times mm$	—	—	—	25	21	46	24	26	50	1:1	1:1.08
$F_2 D_8 \text{♀} \times F_1 D_2 \text{♂}$	$Sm \times Mm$	16	24	40	13	10	23	11	11	22	2:1:1	1.8:1:1

Hence these blotched moths, although heterozygous for their own colour, are certainly not so for melanism.

Although five-sixths of the first-mentioned batch emerged during the winter, enough examples were reared in the succeeding spring to enable the enquiry to be pushed further; unfortunately such was not possible with the second family  $F_2 D_8 \text{♀} \times F_1 D_2 \text{♂}$ .

Bearing in mind that the former lot had the parentage  $F_2$  (*crepuscularia* var. *delamerensis* ♀ × *bistortata* ♂) ♀ × *bistortata* ♂, let us denote it for ease in reference as  $E$  in the typical insects, and as  $E_1$  in the streaks. Then, on the basis of this notation, the following new pairings were made; viz.  $E \text{♀} \times E \text{♂}$ ,  $E_1 \text{♀} \times E \text{♂}$ ,  $E \text{♀} \times E_1 \text{♂}$ ,  $E_1 \text{♀} \times E_1 \text{♂}$  (two families),  $E_1 \text{♀} \times (e \text{♀} \times D_2 \text{♂}) \text{♂}$  and, lastly,  $E_1 \text{♀} \times$  heterozygous *crepuscularia* var. *delamerensis* ♂ from Staffordshire. In every instance fertile ova were secured, and the larvae from them, owing to their early emergence, fed up on hawthorn throughout, beginning when the leaves were young and tender and ending when they were old and tough. Little loss was experienced as the experiment proceeded, and the pupae obtained were followed in due course by the imagines. Table X contains the essential data concerning these.

As can be seen, the facts are in full accordance with the hypothesis that we are concerned with a series of multiple allelomorphs,  $M$ ,  $S$ ,

<sup>1</sup> Since the first specimen I reared was streaked rather than blotched I prefer to designate the character as "streak," thereby avoiding any ambiguity when one expresses the allelomorphic series symbolically.

TABLE X.

To show the inheritance of streak.

Family	Type of Mating	Melanics			Streaks			Types			Expected Ratio	Actual Ratio
		Males	Females	Totals	Males	Females	Totals	Males	Females	Totals		
$E \text{ } \varnothing \times E \text{ } \sigma$	$nm \times nm$	—	—	—	—	—	—	53	62	115	{ All types	{ All types
$E_1 \text{ } \varnothing \times E \text{ } \sigma$	$Sm \times nm$	—	—	—	27	30	57	32	28	60	1:1	1:1.05
$E \text{ } \varnothing \times E_1 \text{ } \sigma$	$nm \times Sm$	—	—	—	46	41	87	37	43	80	1:1	1:1.9
$E_1 \text{ } \varnothing \times E_1 \text{ } \sigma$	$Sm \times Sm$	—	—	—	29	25	54	7	12	19	3:1	2.8:1
$E_1 \text{ } \varnothing \times E_1 \text{ } \sigma$	$Sm \times Sm$	—	—	—	40	33	73	8	10	18	3:1	4:1
$E_1 \text{ } \varnothing \times (e \text{ } \varnothing \times D_5 \text{ } \sigma)$	$Sm \times Mm$	24	12	36	8	10	18	12	4	16	2:1:1	2:1:9
$E_1 \text{ } \varnothing \times \text{del. } \sigma$	$Sm \times Mm$	29	32	61	14	18	32	10	9	19	2:1:1	2:1:6

and *m*, corresponding to the melanic, streak, and type characters respectively. To secure further proof of its validity new pairings were made—a procedure rendered the more urgent by the presence in family ( $E_1 \text{ } \varnothing \times \text{crepuscularia}$  var. *delamerensis*  $\sigma$ ) of moths so pale that I described them in my notes as “nearly” types. The parentages of these additional cultures were as follows:

- (I) “Nearly” type  $\varnothing$  ( $ex E_1 \text{ } \varnothing \times cd \text{ } \sigma$ )  $\times$  type  $\sigma$  (same origin).
- (II) Streak  $\varnothing$  ( $ex E_1 \text{ } \varnothing \times E \text{ } \sigma$ )  $\times$  Streak  $\sigma$  (same origin).
- (III) Type  $bi^d \text{ } \varnothing \times$  Streak  $\sigma$  ( $ex E_1 \text{ } \varnothing \times E_1 \text{ } \sigma$ ).
- (IV)  $bi^d \text{ } \varnothing \times$  Streak  $\sigma$  ( $ex E_1 \text{ } \varnothing \times E \text{ } \sigma$ ).
- (V)  $bi^d \text{ } \varnothing \times$  Melanic  $\sigma$  ( $ex E_1 \text{ } \varnothing \times cd \text{ } \sigma$ ).
- (VI) Melanic  $F_1$  ( $cd \text{ } \varnothing \times bi^d \text{ } \sigma$ )  $\varnothing \times (E_1 \text{ } \varnothing \times cd \text{ } \sigma) \text{ } \sigma$ .
- (VII) Type  $\varnothing$  ( $ex E_1 \text{ } \varnothing \times E_1 \text{ } \sigma$ )  $\times$  Streak  $\sigma$  ( $ex E_1 \text{ } \varnothing \times E \text{ } \sigma$ ).
- (VIII) Melanic  $\varnothing$  ( $ex E_1 \text{ } \varnothing \times cd \text{ } \sigma$ )  $\times$  Melanic  $\sigma$  (same origin).
- (IX) [ $\{bi^d \text{ } \varnothing \times (cd \text{ } \varnothing \times bi^d \text{ } \sigma) \text{ } \sigma\} \text{ } \varnothing \times bi^d \text{ } \sigma$ ] type  $\varnothing \times$  Streak  $\sigma$  ( $ex E_1 \text{ } \varnothing \times E_1 \text{ } \sigma$ ).
- (X) Type  $\varnothing$  ( $ex E \text{ } \varnothing \times E_1 \text{ } \sigma$ )  $\times$  Streak  $\sigma$  ( $ex E_1 \text{ } \varnothing \times E \text{ } \sigma$ ).
- (XI) Streak  $\varnothing$  ( $ex E \text{ } \varnothing \times E_1 \text{ } \sigma$ )  $\times$  Melanic  $\sigma$  ( $ex E_1 \text{ } \varnothing \times cd \text{ } \sigma$ ).
- (XII)  $bi^d \text{ } \varnothing \times$  type  $\sigma$  ( $ex E_1 \text{ } \varnothing \times E \text{ } \sigma$ ).
- (XIII) Type  $\varnothing$  ( $ex E_1 \text{ } \varnothing \times E \text{ } \sigma$ )  $\times$  type  $\sigma$  ( $ex E \text{ } \varnothing \times E \text{ } \sigma$ ).
- (XIV)  $bi^d \text{ } \varnothing \times$  Streak  $\sigma$  ( $ex$  IV).
- (XV) Streak  $\varnothing$  ( $ex$  XIV)  $\times bi^d \text{ } \sigma$ .
- (XVI) Type  $\varnothing$  ( $ex$  XIV)  $\times bi^d \text{ } \sigma$ .
- (XVII) Type  $\varnothing$  ( $ex V \text{ } \varnothing \times cr \text{ } \sigma$ )  $\times$  Melanic  $\sigma$  ( $ex E_1 \text{ } \varnothing \times cd \text{ } \sigma$ ).
- (XVIII) [ $bi^d \text{ } \varnothing$  (Kent)  $\times bi^d \text{ } \sigma$  (Hants)]  $\varnothing \times$  Streak  $\sigma$  ( $ex$  IX).
- (XIX) Streak  $\varnothing$  ( $ex$  XVII)  $\times \{bi^d \text{ } \varnothing$  (Kent)  $\times bi^d \text{ } \sigma$  (Hants)]  $\sigma$ .
- (XX) Streak  $\varnothing$  ( $ex$  XIV)  $\times \{bi^d \text{ } \varnothing$  (Kent)  $\times bi^d \text{ } \sigma$  (Hants)]  $\sigma$ .
- (XXI) Type  $\varnothing$  ( $ex E_1 \text{ } \varnothing \times E \text{ } \sigma$ )  $\times$  type  $\sigma$  ( $ex q$ ).

Again the broods attained maturity but in this case, since hawthorn was then hopelessly unpalatable, the substitute food, knotgrass, was supplied to the larvae. Table XI contains all the necessary information as to the wing colour and sex of the various families, and offers full confirmation of the hypothesis, just put forward, on the genetical relation of "streak" to melanic and type. It also shows, incidentally, that the "nearly" type melanics behaved genetically like their fully pigmented relatives.

TABLE XI.

*Additional families showing inheritance of streak.*

Family	Type of Mating	Melanics			Streaks			Types			Expected Ratio	Actual Ratio
		Males	Females	Totals	Males	Females	Totals	Males	Females	Totals		
I	<i>Mm</i> × <i>mm</i>	11	7	18	—	—	—	5	12	17	1 : 1	1 : 1
II	<i>Sm</i> × <i>Sm</i>	—	—	—	31	22	53	12	6	18	3 : 1	2.9 : 1
III	<i>mm</i> × <i>SS</i>	—	—	—	40	45	85	—	—	—	All streaks	All streaks
IV	<i>mm</i> × <i>Sm</i>	—	—	—	34	27	61	35	32	67	1 : 1	1 : 1.1
V	<i>mm</i> × <i>MS</i>	33	15	48	31	23	54	—	—	—	1 : 1	1 : 1.1
VI	<i>Mm</i> × <i>MS</i>	54	42	96	17	17	34	—	—	—	3 : 1	2.8 : 1
VII	<i>mm</i> × <i>Sm</i>	—	—	—	14	15	29	17	14	31	1 : 1	1 : 1.1
VIII	<i>MS</i> × <i>MS</i>	14	16	30	5	7	12	—	—	—	3 : 1	2.5 : 1
IX	<i>mm</i> × <i>Sm</i>	—	—	—	25	24	49	22	16	38	1 : 1	1.3 : 1
X	<i>mm</i> × <i>Sm</i>	—	—	—	4	7	11	8	9	17	1 : 1	1 : 1.5
XI	<i>Sm</i> × <i>Mm</i>	11	11	22	5	7	12	5	3	8	2 : 1 : 1	2 : 1.1 : .7
XII	<i>mm</i> × <i>mm</i>	—	—	—	—	—	—	46	49	95	All types	All types
XIII	<i>mm</i> × <i>mm</i>	—	—	—	—	—	—	32	27	59	All types	All types
XIV	<i>mm</i> × <i>Sm</i>	—	—	—	25	24	49	34	25	59	1 : 1	1 : 1.2
XV	<i>Sm</i> × <i>mm</i>	—	—	—	15	11	26	12	11	23	1 : 1	1 : 1.1
XVI	<i>mm</i> × <i>mm</i>	—	—	—	—	—	—	43	35	78	All types	All types
XVII	<i>mm</i> × <i>MS</i>	11	17	28	17	15	32	—	—	—	1 : 1	1 : 1.1
XVIII	<i>mm</i> × <i>Sm</i>	—	—	—	8	7	15	6	6	12	1 : 1	1.2 : 1
XIX	<i>Sm</i> × <i>mm</i>	—	—	—	10	10	20	11	8	19	1 : 1	1 : 1
XX	<i>Sm</i> × <i>mm</i>	—	—	—	9	13	22	14	14	28	1 : 1	1 : 1.25
XXI	<i>mm</i> × <i>mm</i>	—	—	—	—	—	—	49	41	90	All types	All types

Nevertheless, to obtain further evidence, a streak male, taken from brood IV, was in turn mated back on *T. bistortata*. This once more gave rise to streaks and types in equal numbers. This procedure was repeated a fifth time by mating a streak from this last-named batch with a type male bred from a cross between two southern races (from Kent and Hampshire respectively) of *bistortata*, when again the same ratio arose.

Next season this was carried out a sixth, and yet a seventh time, with exactly the same result. Not only was this so, but in addition, types chosen from the former family were mated *inter se*, and so were

streaks. Moreover, streaks were paired with types of the same origin, with typical *T. crepuscularia*, and with a female of the  $F_1$  cross between *crepuscularia* (type) female and *bistortata* male. A glance at Table XII will disclose the condition of the batches bred.

TABLE XII.

Family	Type of Mating	Streaks			Types			Expected Ratio	Actual Ratio
		Males	Females	Totals	Males	Females	Totals		
XXII									
(XX ♀ × <i>bist.</i> ♂) ...	<i>Sm</i> × <i>mm</i>	32	27	59	28	25	53	1 : 1	1.1 : 1
XXIII									
(XXII ♀ × <i>bist.</i> ♂)...	<i>Sm</i> × <i>mm</i>	14	15	29	19	15	34	1 : 1	.86 : 1
XXIV									
(XX ♀ × <i>crep.</i> ♂) ...	<i>Sm</i> × <i>mm</i>	10	4	14	9	2	11	1 : 1	1.2 : 1
XXV									
(XXII ♀ × XXII ♂)	<i>mm</i> × <i>mm</i>	—	—	—	59	50	109	{ All types	{ All types
XXVI									
(XXII ♀ × XXII ♂)	<i>Sm</i> × <i>Sm</i>	26	22	48	10	7	17	3 : 1	2.82 : 1
XXVII									
(XXII ♀ × XXII ♂)	<i>mm</i> × <i>Sm</i>	17	19	36	19	13	32	1 : 1	1.1 : 1
XXVIII									
$F_1$ ( <i>cr.</i> ♀ × <i>bist.</i> ♂) ♀ × XXII ♂	<i>mm</i> × <i>Sm</i>	27	21	48	29	16	45	1 : 1	1 : 1

Thus, after no less than seven<sup>1</sup> "passage infections," as it were, the streak pattern emerges just as it was originally introduced, and exhibits normal Mendelian behaviour throughout.

#### CONCLUDING REMARKS.

We have discovered, during the work described in the foregoing pages, that new experiments, intended to cast light on abnormalities in the inheritance of melanism in interspecific crosses between *Tephrosia bistortata* and *T. crepuscularia* var. *delamerensis*, have themselves given rise to results comparable with the earlier ones yet not the same. In the 1920 work, two  $F_2$  generations of *crepuscularia* × *bistortata* hybrids included a long range of nondescript insects, or rather insects only describable as individuals. In the latest series, however, whilst we still have an apparent failure of clean cut segregation, the variation is far from being so chaotic. Instead, there has developed in a number of the  $F_2$  *crepuscularia* var. *delamerensis* ♀ × *bistortata* ♂ hybrids, which ought to have been melanics, an extremely curious modification in

<sup>1</sup> Another brood, reared by pairing a female streak from family XXIII with a *bistortata* ♂, attained maturity in July 1923 and yielded 17 streaks and 20 types.

wing colour and pattern. Sharply contrasting with this, except perhaps that in occasional broods, types manifested themselves in greater numbers than theory demanded, the crosses originating with *bistortata* females and *crepuscularia* males reveal melanism acting as a simple Mendelian dominant. Such, too, is the state of affairs in the back crosses in which an  $F_1$  (*crepuscularia* var. *delamerensis* ♀ × *bistortata* ♂) male takes part, and in broods of more or less complex type derived from the true melanics in the  $F_2$  generation of the same parentage. These several lots, yielding results in perfect harmony with one's anticipations, need not detain us longer.

On the contrary, the occurrence of streaked and blotched forms calls for very careful treatment, and this we shall commence by considering the insects with which the research began.

Since "streak" was found to be dominant over type, and recessive to black, two facts are clear at once: (1) that it could not be inherited from type insects, (2) that the melanic parent might have been heterozygous for streak. That the latter was not the case was demonstrated by the long series of crosses made between types and brothers and sisters of the black parent. The offspring of such unions was invariably melanic. Hence their dark parents and their sister were homozygous for melanism, and streak did not therefore enter into their genetic constitution.

In dealing with the original experiments, I insisted that the observed conditions arose through accidents and incidents in gametogenesis induced by the circumstance that we were studying hybrids in the wider sense, i.e., those produced by mating parents appertaining to distinct Linnaean species. Unhesitatingly, I assign the presence of streaked, or rather of mosaic insects, in the affected  $F_2$  broods to the same causes, so that no necessity arises for the repetition of the arguments employed there.

We seem compelled to admit that the new character must have originated from some change in the determiner for melanism, and this in itself ought to make us pause before placing it in the colour series of allelomorphs. Let us assume, therefore, that despite the breeding evidence, "streak" is not a genuine member of such a group of multiple allelomorphs. On that basis several explanations of its nature lie open, and these we shall proceed to discuss one by one.

(1) Explanations may be sought on the simple basis of a possible contamination of the gene for melanism brought about during contact with that for its allelomorph.



If gametic contamination accounted in full for the circumstances, then difficulties at once present themselves. In particular, the condition of the later broods, coupled with the fact that streak, after seven crossings with typical *bistortata*, emerged as it entered, shows that such contamination must have ceased with the gametogenesis of the  $F_1$  insects and stabilised itself at the grade then attained. Furthermore, against the correctness of that view many forceful arguments can be employed. If the gene for melanism has been modified by contact with its allelomorph it seems strange, firstly, that the action was not reciprocal, as the advent of types in their wonted numbers conclusively proved, secondly, that a relatively small number of similar determiners was affected, and, lastly, that similar genes in the *bistortata* ♀ × *crepuscularia* var. *delamerensis* ♂ batches exhibited no parallel change.

In any case, if this be the actual basis of streak, the position we have reached is that a third member falling into a series of recognised allelomorphs may arise in such a way, and the bedrock fact, that it is an allelomorph, is not altered in the slightest.

(2) Modifying factors capable of destroying the purity of the melanism secured by the action of the main gene may be at work.

Such factors as are here postulated may conceivably have originated in different ways. They may have arisen *de novo* during hybrid gametogenesis, or they may have been complementary, or other ones brought into collaboration in one of the new combinations rendered possible by hybridisation. Since either origin is possible and neither can be definitely proved or disproved, the matter must be argued out in a general sort of manner. But let us be careful at this stage to note that, if the modifiers are quite new, then it is equally feasible for us to regard a single determiner for streak as new—a much more likely position.

Be the origin of these hypothetical determiners what it may, we have two additional possibilities to take into consideration. The accessory genes may be linked to that for melanism or they may act quite independently. If they segregate irrespective of the course taken by the main factor then, by crosses with typical insects selected from various sources, one ought to be able to detach them. In that event, sooner or later, pure melanics should crop up. Since black insects have never put in an appearance, although many broods of the nature stipulated have been bred, the notion of such independent modifiers may be dismissed.

On the other hand, if the suggested modifiers be linked directly to the chief factor, or the two act in unison with some common structure,



then their detection, or definite proof of their absence, would prove exceedingly difficult. Should, however, the strength of the linkage, although very great, be not perfect then, through the influence of some agency like the process known familiarly as "crossing-over," whenever the principal gene for melanism separated from its modifiers, melanics should occur. Again, one can only urge that never, throughout an abundance of broods, have such unexpected melanics appeared. Thus, once more, we are reduced to regarding the position of streak as a new allelomorph or something indistinguishable therefrom.

(3) A loss of a distributing factor, the effect of which was to secure the even distribution of the black pigment, would account for the facts.

In this case, too, such a factor may be independent or linked with that for melanism. In the former event, then, breeding experiments of many types would have led previously to its detachment and detection; in the latter, under ordinary circumstances, the linkage must have been absolute, for no ordinary intraspecific pairings between types and melanics have ever served to separate the two. Only the far-reaching effects of interspecific hybridity seem to have been potent enough to bring about the excessively rare "crosses-over" responsible for the manifestation of a streak. In any case the position is indistinguishable from that of the presence of a new allelomorph.

(4) The development of streak may, conceivably, be the phenotypical expression of the non-disjunction of the determiners for wing coloration in the  $F_1$  gametogenesis, with subsequent irregularities in somatic mitoses in  $F_2$  and later insects.

In the gametogenesis of the *crepuscularia* var. *delamerensis* ♀ × *bistortata* ♂ hybrids we know, from cytological examination, that imperfect reduction divisions occur in which certain of the chromosomes pass undivided to one pole of the maturation spindle. If the chromosomes acting in this fashion include the homologous pair carrying the genes for wing colour, then there arises a case of non-disjunction leading to the production of gametes bearing such chromosomes in duplicate. As far as these genes are concerned, therefore, they may be regarded as being of the composition  $Mm$ . Such gametes may fuse in fertilisation with the further trio,  $M$ ,  $m$  and  $Mm$  (all of which are of possible occurrence in such  $F_1$  hybrids), thereby yielding the zygotes  $MMm$ ,  $Mmm$  and  $MMmm$ . If mitotic irregularities take place in the somatic cells of such zygotes, through the mechanical interference of the massed homologues, so that somatic non-disjunction occurs, then mosaics like our streaks would result. But,

in this event, recognising that not a single portion of the external surface of the affected insects is free from the mosaic effect, we must grant that the internal cells likewise will be subject to the same phenomenon. With such cells we must class the oogonia and spermatogonia. If we admit this, as seems reasonable, then sooner or later, in greater quantity or small, gametes purely of type *M* are bound to arise so that in the next cross with type insects melanics should appear. However, when we examine the many examples of such broods open to inspection, we perceive, as was pointed out before, that not a single family includes a melanic. Hence, unless the behaviour of the homologous chromosomes is perfect in the primordial germ cells and irregular in those of the soma—an almost inconceivable<sup>1</sup> position—non-disjunction fails to account satisfactorily for the presence of the streaks, or for the regularity of the inheritance of the form.

To sum up, all of the above hypotheses seem more cumbrous than the one of assuming that the unit character melanism has been so changed, and changed permanently, that we have now a third allelomorph to add to the former pair of melanic and type. To conclude otherwise would be to introduce complexity where simplicity suffices.

Granting this, then the gene in question produces effects similar to those responsible for piebald patterns studied in guinea pigs by Ibsen and in rabbits by Castle, and to those investigated by Baur and others, giving rise to variegation in *Lychnis*, *Antirrhinum* and *Aquilegia* amongst the plants. Never at any stage do the experiments give indications of parallelism in genetic behaviour between "streak" in my insects and "patch" in Punnett's sweet peas.

There remain now for discussion the last two families in Table V E. In the first of these an  $F_1$  (*crepuscularia* var. *delamerensis* ♀ (homozygous) × *bistortata* ♂) female, apparently a melanic, and therefore of formula *Mm*, was mated to a pure *bistortata* male certainly of constitution *mm*. According to Mendelian expectation their progeny should

<sup>1</sup> In connection with this, however, it must be remembered, as demonstrated by Hegner and others, that, in the early cleavage stages of certain insects like *Miastor* and *Chironomus* the cleavage nucleus or nuclei encountering the granules of the pole disc, or the so-called germ cell determinants, segregate as the primordial germ cells, and again that such germ cells, and those arising from them, are not subject to the chromatin diminution process observed in those nuclei destined to give rise to the blastoderm layer. In other words, it may be possible that the function of the germ cell determinant is to regulate as far as possible the behaviour of the chromosomes, and that, therefore, differences in behaviour in the matter of non-disjunction in the descendants of the two sets of cells are to be regarded as within the bounds of possibility.

include equal numbers of types and heterozygous melanics. Instead, we have 106 types and, in addition, 1 melanic and 1 mosaic or streak. In the other instance we observe a diametrically opposed phenomenon; a female of similar parentage (save that the original *crepuscularia* var. *delamerensis* female had been heterozygous for melanism), and seemingly a type of build *mm*, crossed with a *bistortata* male, likewise of composition *mm*, produced 56 melanics and 19 types in place of the anticipated wholly type brood.

How can such anomalies have arisen? Considering the ratio of melanics to types in family *D*, ♀ × *bistortata* ♂ we seem rather to be concerned with the ratio obtained in a dihybrid back cross than with a monohybrid ratio, i.e. it approximates 3:1 instead of 1:1. In this fact seems to lie the solution of the problem, for, comparing these figures with those given by Nilsson-Ehle in his work on grain colour, or with Shull's for duplicate genes for capsule form in *Capsella bursa-pastoris*, and taking due cognisance of the point that ours is a back cross, we have at once impressed on us the fact that the workings of a system of duplicate genes for wing colour would account for our observations.

But again it will be urged, how can this be when, phenotypically, the female parent, in whose ancestry melanism alone occurs, was a type? Let us suppose that, at the first cleavage division in the fertilised egg from which this female proceeded, the chromosome bearing the gene for melanism, after division, so behaved that the split halves were included in one daughter nucleus. The immediate effect would be to generate nuclei incapable of setting up melanism as well as others carrying the determiners for that character in duplicate. If the former gave rise to the blastomeres, and the latter to the primordial germ cells, then we should possess a zygote phenotypically a type but genotypically of melanic powers. Not only is this insect thus constituted germinally but, in addition, the nuclei of its germ cells before maturation would be endowed with three homologous chromosomes, two bearing the gene for melanism and the other without. On gametogenesis this insect would produce the gametes *MM*, *Mm*, *M* and *m*, the actual numbers of each depending upon the path pursued by the odd chromosome, and whether or not it divides. In any event, we should have an excess of gametes capable of inducing melanism, and that despite the fact that the insect developing them is phenotypically a type. Any moth so constituted, when paired with ordinary *bistortata*, must yield a large excess of melanics precisely as seen in the experiment.

And in the foregoing, I think, lies the correct explanation of the family  $D_6 \text{ ♀} \times \textit{bistortata} \text{ ♂}$ , and of the genetic behaviour of Castle's non-transmissible tricolour pattern in rats.

The very tempting assumption that we have here an extreme phase of the mosaic condition must be cast aside, for such an insect would be genetically a mosaic as breeding tests with "almost" type mosaics conclusively prove. For the phenomena seen in family  $D_6 \text{ ♀} \times \textit{bistortata} \text{ ♂}$  the same explanation seems to be adequate except that the mitotic dislocation postulated by the scheme must be of the reverse order, i.e. that the cleavage nuclei taking part in blastoderm formation must have been descended from those in which the genes for melanism were duplicated, or otherwise, that the chromosome proceeding abnormally, or occurring in duplicate in the primordial germ cells, must have been the homologue void of such determiners.

If, as seems likely, this supplies a correct view of the second brood, then the presence of a melanic, as well as of a mosaic, proves that the accident giving rise to the circumstances described must have occurred at a period allowing of the inclusion within the segregating primordial germ cells of a few, or at least one, of the cleavage nuclei furnished with the genes for melanism.

This view affords indirect evidence, that mitotic abnormalities admitting of the duplication of homologous chromosomes, and therefore of genes, cannot account for the advent of streaks; and, we are driven to look for their origin in a direct change in the determiner for melanism itself.

#### SUMMARY.

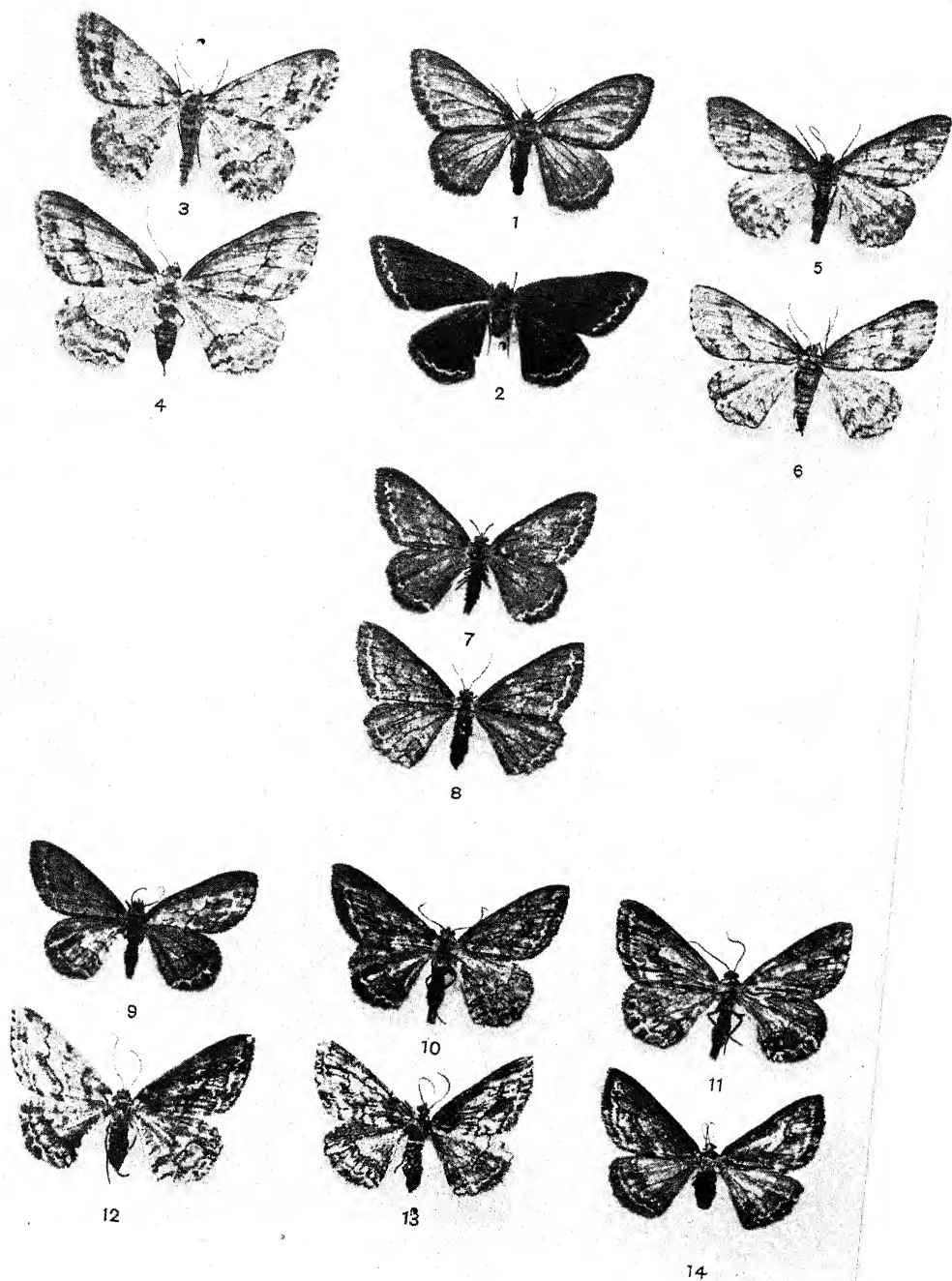
(1) Further investigations into the inheritance of melanism in the interspecific crosses between *Tephrosia crepuscularia* var. *delamerensis* female and *T. bistortata* male reveal, as previously, a failure in the 3:1 ratio in the  $F_2$  generation of such hybrids.

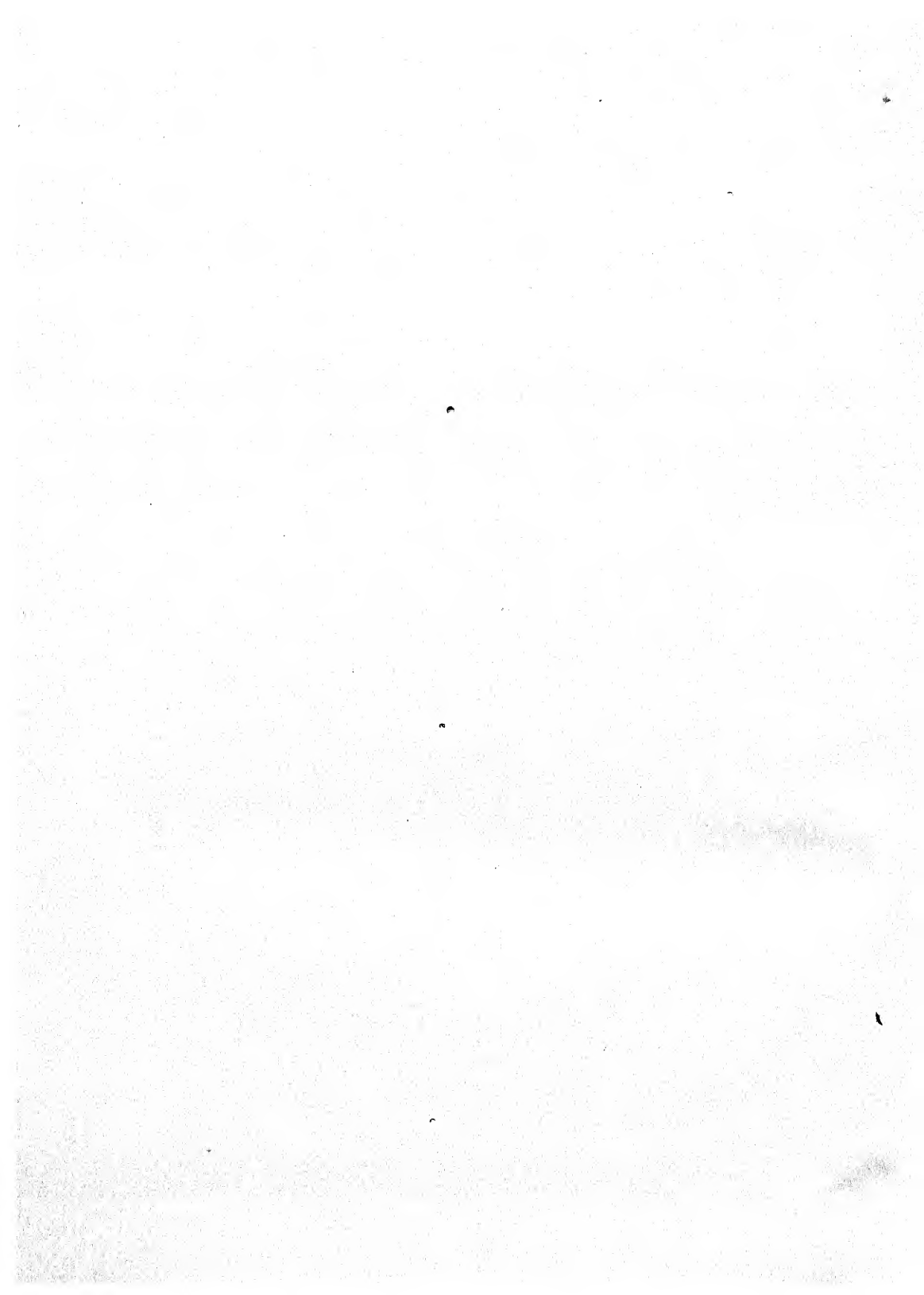
(2) Unlike what occurred in the early cross, no great spread of variation was encountered in the  $F_2$  insects.

(3) Instead of types and melanics the  $F_2$  brood included types, streaks (mosaics) and melanics.

(4) The character "streak," when tested by means of breeding experiments, proved to form, with melanism and type, a series of multiple allelomorphs with three members.

(5) Streak is dominant to type and recessive to melanism.





(6) This character originated through a degradation of the gene for melanism during interspecific crossing.

(7) Unit-characters are therefore not constant under all conditions.

(8) In the cross between *bistortata* female and *crepuscularia* var. *delamerensis* male, and others in which this hybrid was concerned, in back crosses and other broods involving  $F_1$  melanic (*crepuscularia* var. *delamerensis* ♀ × *bistortata* ♂) males, and in complex crosses in which the true melanics of the  $F_2$  (*crepuscularia* var. *delamerensis* ♀ × *bistortata* ♂) participated, the inheritance of melanism was of the usual simple Mendelian type.

(9) Certain back crosses between (*crepuscularia* var. *delamerensis* ♀ × *bistortata* ♂) females and *bistortata* males gave rise to extremely aberrant ratios.

### EXPLANATION OF PLATE XVIII.

Figs. 1 and 2. Male and female *T. crepuscularia* var. *delamerensis*. (The female is the ancestress of all the "streak" broods.)

Figs. 3 and 4. Male and female bivoltine *T. bistortata*, 1st brood.

Figs. 5 and 6. " " " " 2nd brood.

Fig. 9. Male "streak" from family  $F_2 D_8$ .

Fig. 10. " " "  $F_1 D_8 \times T. bistortata$  ♂.

Fig. 11. " " "  $E_1 \text{♀} \times E \text{♂}$ .

Fig. 12. Female "streak" from family IV.

Fig. 13. " " " XXIII.

Fig. 14. " " " XXVI.

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THE PHENOMENON OF MUTUAL AVERSION  
BETWEEN MONO-SPORE MYCELIA OF THE  
SAME FUNGUS (*DIAPORTHE PERNICIOSA*,  
MARCHAL). WITH A DISCUSSION OF SEX-  
HETEROTHALLISM IN FUNGI.

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(With Plates XIX, XX and Three Charts.)

*Preliminary Note.*

DURING the investigation into the life history of *Diaporthe pernicios*a (Marchal), a fungus which gives rise to a rapid wilt and "die-back" of stone fruits in this country, a new phenomenon has been observed, namely that of mutual aversion of certain monospore cultures of this organism when grown together on artificial media. So far as is known to the writer, this phenomenon of aversion between colonies of the same fungus has not been recorded hitherto<sup>1</sup>, and although the investigation is far from complete, the results, as far as they go, were thought to be of sufficient interest to allow of the publication of this preliminary note.

*Diaporthe pernicios*a was first isolated by Marchal(18) while working on rots of stored fruits in Belgium, and an organism, morphologically similar, has been found to be one of the causal organisms of wilt and "die-back" of stone fruit trees in this country. A specimen of "die-back" and a pure culture of *Diaporthe* from plum were submitted to Professor Marchal, and he is of the opinion that the two organisms are the same. Inoculation experiments have shown that *Diaporthe* from stone fruits can cause rot of apples very similar to the rot caused by a pure culture of *Diaporthe* obtained from Professor Marchal.

<sup>1</sup> Since the above was in manuscript a paper by Dodge(10) has been brought to my notice in which he mentions that mono-spore strains of *Ascobolus magnificus* of the same sex, and also pieces of mycelium of the same mono-spore strain when combined together will not meet, and "there is a narrow region which remains comparatively free from hyphae as though there was antagonism or repulsion between the two."

The observations recorded in this communication were made on *D. perniciosus* isolated from diseased tissues of stone fruit trees.

*D. perniciosus* is an Ascomycete, belonging to the Valsaceae group of the Sphaeriales, and has two distinct stages, (1) the pycnidial or *Phomopsis* stage and (2) the perithecial or *Valsa* stage<sup>1</sup>.

The pycnidia are formed under the bark of the host, and contain two kinds of spores, the oval viable "a" spores, as designated by Diedicke (9) and the filiform, non-viable, or "b" spores.

There is considerable divergence of opinion as to whether the "b" spores are typical spores, or only paraphyses or "basides" as held by Marchal. Repeated attempts to germinate these "b" spores have failed, and as far as can be seen they do not appear to have any definite nucleus. In *D. perniciosus* the "b" spores are formed first, later both "a" and "b" spores are present in the same pycnidium and finally "a" spores only.

The perithecia either develop in the same stroma as the pycnidia, or independently in the more deeply seated cortical tissues of the host. The perithecia contain numerous asci with eight bicellular ascospores.

The pycnidia and the necks of the perithecia burst through the bark, forming lenticel-like slits.

Considerable difficulty has been experienced in inducing *D. perniciosus* to pass through all the stages of its life cycle in pure culture on an artificial medium. Cultures from mass infections with pycnospores, and mono-pycnospore cultures develop the typical pycnidial stage with "a" and "b" spores very readily on a number of different media, but typical perithecia have only been found in three pycnospore cultures, one of which was a mono-spore culture, SS<sub>1</sub>, and then only sparingly after many months' growth. Inoculations with this mono-spore culture have given rise to typical "die-back" of plum, and this strain has developed perithecia freely on the host plant.

Ascospore cultures, on the other hand, have given very variable results. Among mass platings of ascospores, some gave the typical pycnidial stage with "a" and "b" spores, others developed perithecia only. Mono-ascospore cultures develop perithecia in large numbers, but few or no pycnidia, and if pycnidia occur at all they contain rather undersized oval spores; no filiform spores have been found in mono-ascospore cultures.

It was concluded from these results that *D. perniciosus* might be

<sup>1</sup> A full description of the morphological characters of the fungus is given in a paper on "die-back" disease of stone fruits. *Annals of App. Biol.* Vol. x. No. 2, 1923.

heterothallic, two strains being necessary to enable the fungus to complete all the stages of its life history in pure culture.

A considerable number of mono-ascospore cultures were then isolated.

### *Technique.*

The technique employed for the isolation of all the mono-spore cultures was that elaborated by Carl de la Rue (8) as follows: Sufficient 30 % gelatine is poured into a petri dish to form a thin film about 1 mm. deep and allowed to solidify. Then five or six drops of sterile distilled water are placed at intervals on the surface of the gelatine. The drops of water should roll on the surface if the gelatine is in the right condition of firmness. A platinum loop-full of a dilute suspension of spores is then placed in each drop, and the drops spread thoroughly over the surface of the gelatine with a sterile glass spreader. A dummy objective marker, as designed by C. de la Rue, with an aperture of a 1/6 objective, is then fixed to the triple nose-piece of the microscope, after having been previously dipped in alcohol and flamed. The gelatine plate can be examined under a low power, and when a field is found containing a single spore, the nose-piece is swung round and the marker gently pressed into the gelatine. The field is then re-examined to see that no other spores are present in the circle marked, before the circle is picked out under a lens with a small sterile platinum loop and transferred to a slope of culture medium. The dummy objective must be flamed and allowed to cool between each spore.

When the mono-spore cultures have grown, pieces of mycelium of two, or at the most three, separate cultures can be placed on a plate of solid culture medium and grown on together in an incubator.

In some combinations it was found that the resulting colonies grew across the plate and intermingled freely (Pl. XIX, figs. 1, 3, 4, 7); but others showed a definite aversion, leaving a well-defined line of demarcation between the colonies (Pls. XIX and XX). In the latter case the growth of the surface mycelium is arrested along the line of demarcation, and although at first the hyphae submerged in the medium intermingle, later they become disorganised and finally die out. A thick-walled stromatic protective growth of mycelium forms at the edge of each colony on either side along the line of demarcation, in some cases accompanied by discoloration of the medium (Pl. XX, fig. 9). After a time the agar dries out and cracks along the line.

If two pieces of mycelium from one and the same averting mono-spore strain are tested together, the colonies will intermingle and show

no aversion (Pl. XIX, figs. 1, 3). If two or three different mono-spore mycelia are grown in the same plate, they may either meet—if all the strains are alike—(Pl. XIX, figs. 1, 3, 4, 7); or, two will meet and a third show aversion to the other two (Pl. XIX, figs. 5, 6, 8, 9); or, in some cases, there will be a general aversion between all three colonies (Pl. XX, figs. 2-6).

Since two pieces of mycelium from the same strain will meet when grown together on the same plate, although that strain has shown aversion towards other strains, and since one colony on a plate can show aversion towards two other colonies which meet, all three of which have been isolated from the same host, like must meet like, and unlike show aversion to unlike. For the same reasons, the line of demarcation cannot be due to staling of the medium, but rather, as the general appearance of the plates suggests, the secretion of some toxin, possibly volatile, by the mycelia. This toxin diffuses more slowly through the substratum, so that the mycelium submerged in the medium is not affected so soon as that on the surface. The nature of the toxin is not known; it shows no reaction to litmus.

The phenomenon of aversion between any two antagonistic strains has occurred on all the media so far used, irrespective of the depth of the medium, the distance at which the inocula are placed, or the age of the cultures when tested. A number of mono-spore cultures have been grown in pure culture for more than two years and subcultured when necessary, on various media, without showing any signs of loss of toxicity.

*D. perniciosa* grows and sporulates well on prune-juice and plum-decoction-agar with or without the addition of crushed oats; on crushed-oats-agar alone, or with the addition of cane sugar or 1% glycerine; also on sterilised plum twigs. After preliminary tests on all the above media had shown that aversion can occur between any two given strains irrespective of the medium, the bulk of the tests have been made on crushed-oats-agar, a medium which has the advantage of being quickly and easily made.

Unfortunately, on account of the small size of the fruiting bodies and other technical difficulties, this fungus does not lend itself to critical cytological investigation; but the perithecium arises from a very definite coiled multicellular archicarp, the cells of which are multinucleate (Pl. XX, figs. 7, 8). No male organ has been found and the archicarp becomes disorganised as the perithecium develops. The early stages of the perithecium resemble those described in *Polystigma rubrum* (2).

After the disorganisation of the archicarp has set in, some of the

smaller hyphae in the interior of the young perithecium show a bi-nucleate condition, but it was not possible to determine whether this bi-nucleate condition had any special significance or whether it was only due to rapid cell division in young actively growing mycelium; neither could it be seen if these hyphae were given off from the multi-nucleate cells of the archicarp. No association of nuclei or pores in the cell walls have been observed in the cells of the archicarp. The formation of ascogenous hyphae and the development of the ascus could not be followed.

As the cytological investigation of this fungus proved to be so difficult and unsatisfactory, the experiment was continued on different lines with the hope of being able to prove by other than cytological means, how and when this segregation or splitting up into physiological strains occurs, whether it is vegetative or promiscuous, or whether it occurs at some definite stage in the life-history of the organism.

*Behaviour of mono-spore mycelia from various sources.*

A total of 319 mono-pycnospore and mono-ascospore cultures have been under observation for some time, and as regards the occurrence of strains on the different hosts the results have shown that aversion may or may not occur; as follows, between :

1. Mono-ascospore } mycelia from different species of host.  
    " -pycnospore }
2. Mono-ascospore } mycelia from different varieties of host.  
    " -pycnospore }
3. Mono-ascospore { mycelia from different perithecia } on the same  
    " -pycnospore {     "     "     "     pycnidia } variety of host, but different trees.
4. Mono-ascospore mycelia from different perithecia on the same host and the same stem.

5. In all perithecia so far tested, no aversion has been observed between mono-ascospore mycelia from the same perithecium, with the exception of one mono-pycnospore strain  $SS_1$ , which developed perithecia. This  $SS_1$  strain will be discussed more fully later (pp. 358, 359, 363).

In the majority of cases, aversion occurs between mono-mycelia from different hosts whether of the same variety, different varieties, or different species, but mono-mycelia isolated from such widely different hosts as apricot and plum have been found to meet; on the other hand averting strains have been found in different perithecia on the same

host (Chart I, Groups VI, VII, VIII), thus showing that the type of strain is irrespective of the variety of host. It is quite possible that the occurrence of averting strains on the same host may be due to multiple infection from two or more different sources, and not to the splitting up into physiological strains in the host plant. More than two strains have not yet been found to occur on the same host, when the spores have been taken direct from diseased tissues, but the results from the investigation of  $SS_1$  have clearly shown that a mono-pycnospor culture is capable of splitting up into a number of averting strains in the first ascospore generation.

But it must be pointed out that although mono-mycelia show aversion when grown on an artificial medium, it does not necessarily follow that this is the case when the parasite is growing on the host. The host may absorb the toxin as soon as it is formed, and the diseased condition may be one of poisoning. There is no mechanical blocking of the vessels by the fungus itself. The mycelium is present chiefly in the medullary rays and cortical tissue. The vessels are frequently blocked by gummy deposits, but not in sufficient numbers to account for the sudden wilting of the whole part affected.

*The development of mono-spore mycelia on artificial media.*

This development can be divided into three distinct classes.

*Class 1.* Mono-pycnospor cultures obtained from pycnidia containing "a" and "b" spores, which appear to be incapable of forming perithecia.

*Class 2.* Mono-ascospore cultures capable of forming a reduced pycnidium with "a" spores only, and numerous mature normal perithecia.

*Class 3.* One mono-pycnospor culture  $SS_1$  (mentioned above) obtained from a pycnidium containing "a" and "b" spores, which produced pycnidia with "a" and "b" spores freely, as in Class 1, but also, after many months, eventually produced mature perithecia with typical 2-celled ascospores. Mono-mycelia obtained from these ascospores have in their turn produced the normal pycnidial stage with both kinds of pycnospores. These cultures have not been growing long enough to show whether they will eventually produce a second generation of perithecia. They may lose their capacity of forming perithecia and revert back to Class 1.

These results are set out in tabular form in Table I.

*Discussion of Table I.*

*Class 1.* This class calls for no further comment, except that as a general rule mono-pycnosporic mycelia from the same pycnidium show no aversion *inter se*, although one definite case of aversion has occurred (Chart III,  $SS_4$  and  $SS_7$ ). The investigation of mono-pycnosporic is not sufficiently complete to allow of any more definite statement. However a number of mono-pycnosporic cultures have been under observation for a considerable time, and have always remained the same whether sub-cultured from spores or pieces of mycelium.

*Class 2.* In this class, which comprises mono-ascospore cultures  $SA_{1-101}$  from different perithecia (Chart I, Groups I-XIII), in no case has aversion been found between mono-ascospore mycelia from the same perithecium. Moreover, the  $F_1$  ascospore generations of the mono-ascospore averting strains  $SA_2$  and  $SA_{19}$  have been fairly extensively tested, and it was found that mono-ascospore strains of the  $F_1$  generation show no mutual aversion *inter se*—whether from the same perithecium or from different perithecia—(Chart I, Groups IX-XIII) or to their respective parents; but the progeny of strain  $SA_2$  shows aversion towards that of strain  $SA_{19}$  (Chart I, Groups IX, X, XI towards Groups XII, XIII), and the progeny of each strain shows aversion towards the parent of the antagonistic strain. Thus, there is no splitting up into averting strains in the  $F_1$  of this class (Pl. XIX).

*Class 3.* In this class, which comprises mono-ascospore cultures  $SA_{108-110}$  and  $SA_{115}-SA_{134}$  and mono-ascus cultures  $SA_{(111)}-SA_{(118)}$  (Chart II, Groups XIV, XV) there is a complete reversal of what occurs in Class 2. Groups XIV, XV are the  $F_1$  ascospore generation of the mono-pycnosporic culture  $SS_1$  already mentioned, which proved capable of forming perithecia in the mono-mycelial condition. Not only is there splitting up into averting strains in the same perithecium (Chart II, Group XV), but the  $F_1$  mono-ascospore mycelia of this strain also shows aversion towards the parent culture (Chart II,  $SS_1$  and Groups XIV, XV).

It will be seen in Chart II that the majority of these mycelia in Groups XIV and XV show aversion *inter se*, but that a few meet; also some of the  $F_1$  mycelia meet one or other of the 10 stock mono-pycnosporic strains with which they have been tested.

If the hypothesis is correct "that like meets like and unlike shows aversion to unlike" any two meeting strains must be the same.



TABLE I.  
*Development of mono-spore mycelia in pure culture.*

Class	Type of fruiting body from which the mono-spore mycelia have been obtained	Fruiting bodies developed by the mono-spore mycelia		Behaviour of the <i>F<sub>1</sub></i> mono-ascospore mycelia from the same fruiting body		Remarks
		Pycnidia with "a" and "b" spores	No perithecia	With the parent	With each other	
1	Pycnidia on bark of host plant containing both "a" and "b" spores 121 <i>mono-pycnospore</i> cultures tested	Pycnidia with "a" and "b" spores	No perithecia	—	—	No <i>F<sub>1</sub></i> ascospore generation
2	Perithecia on bark of host plant or perithecia developed in <i>mono-ascospore</i> cultures 106 <i>mono-ascospore</i> cultures from 13 perithecia	No pycnidia or reduced pycnidia with "a" spores only	Numerous perithecia	No aversion (See Chart I)	No aversion	The <i>F<sub>1</sub></i> <i>mono-ascospore</i> cultures have either no pycnidia, or reduced pycnidia with "a" spores only, but produce numerous perithecia 56 <i>mono-ascospore</i> cultures tested in the <i>F<sub>1</sub></i>
3	<i>SS<sub>1</sub></i> <i>mono-pycnospore</i> culture from a pycnidium on bark of host plant containing both "a" and "b" spores	Pycnidia with "a" and "b" spores	Perithecia	Aversion, but a few meet. (See Chart II)	Aversion, but a few meet. (See Chart II)	The <i>F<sub>1</sub></i> <i>mono-ascospore</i> cultures produce normal pycnidia with "a" and "b" spores. These cultures have not been growing long enough to develop perithecia 90 <i>mono-ascospore</i> <i>F<sub>1</sub></i> cultures tested, of which 64 were isolated from the same perithecium. (See Chart II, Groups XIV, XV)



Chart III shows the behaviour of the 10 mono-pycnospore test strains towards one another.

Until the  $F_2$  of Classes 2 and 3 have been investigated no explanation can be offered to fit the above facts. There certainly appears to be some correlation between the capacity for complete development of the life cycle starting from a single pycnospore and the occurrence of aversion in the same perithecium in the  $F_1$  ascospore generation.

It has not been possible to isolate the eight spores from an individual ascus, to see if segregation occurs in one and the same ascus, but it must be noted that cultures  $SA_{(111), (112), (113), (114)}$  and  $SA_{(115)}$  are mono-ascus cultures (i.e. whole asci) and they behave in the same way as mono-ascospore (i.e. single ascospore) cultures towards other strains. If all the spores of one ascus were not the same, it is conceivable that the degree of aversion would be reduced and the line of demarcation less pronounced. So far no marked difference has been observed, but as this point has not been critically investigated little stress can be laid upon it.

The above results definitely prove that certain mono-spore strains are capable of completing all stages in the life-history of the organism and others not. In the case of *Diaporthe perniciosa*, when dealing with the parasite on the host plant it has been noticed, in some instances, that when there is prolific pycnidial formation the development of a proportionate number of perithecia has not occurred.

These results also throw some light as to when and how the splitting up into physiological strains occurs in the life-history of this fungus, but the proof that certain strains can complete their life-history and others not, and that splitting up into averting strains can occur sometimes, but does not always, leads no further towards the elucidation of the fundamental question as to the constitutional differences of the various strains.

The strains in Class 2 differ from the one strain in Class 3 in that, in the latter class, the parent strain shows aversion to the majority of the mono-mycelia in its own  $F_1$  ascospore generation, whereas in Class 2 the parent meets all mono-mycelia in its  $F_1$ .

This peculiar form of antagonism is quite unlike all known cases of sex-heterothallism in fungi in general, such as in the Mucors (3-7), Ustilaginales (15, 16, 17), Coprini (19, 20), and in *Aleurodiscus* and other Hymenomycetes worked out by Kniep (12-14, 16, 17). In *Diaporthe* it may only be a case of physiological strains and may have nothing to do with sex. The difficulty remains however, as suggested earlier, that antagonistic strains may be able to meet in the host plant. Experiments are

being devised to see if by infecting the same branch with two antagonistic strains an  $F_1$  ascospore generation can be obtained different from that arising from infection with either strain alone.

Cases of sex-heterothallism have been recorded recently in the Ascomycetes. Dodge (10) found in *Ascobolus magnificus* that perithecia would not develop unless two mono-spore mycelia, properly chosen, were combined. This fungus develops male and female organs (archicarp and antheridium), but so far he has not been able to show whether any one mycelium consists entirely of one sex, producing archicarps only and another antheridia only. The mycelia intermix quite freely and numbers of sexual organs are found scattered throughout the hyphal mass, and not only in the region where the mycelia meet. Edgerton (11), working with mono-spore cultures of *Glomerella*, repeatedly isolated + and - strains, which when tested together, formed a dense line of perithecia where the strains met; but he also found that mono-spore mycelia when plated alone were capable of producing a few perithecia.

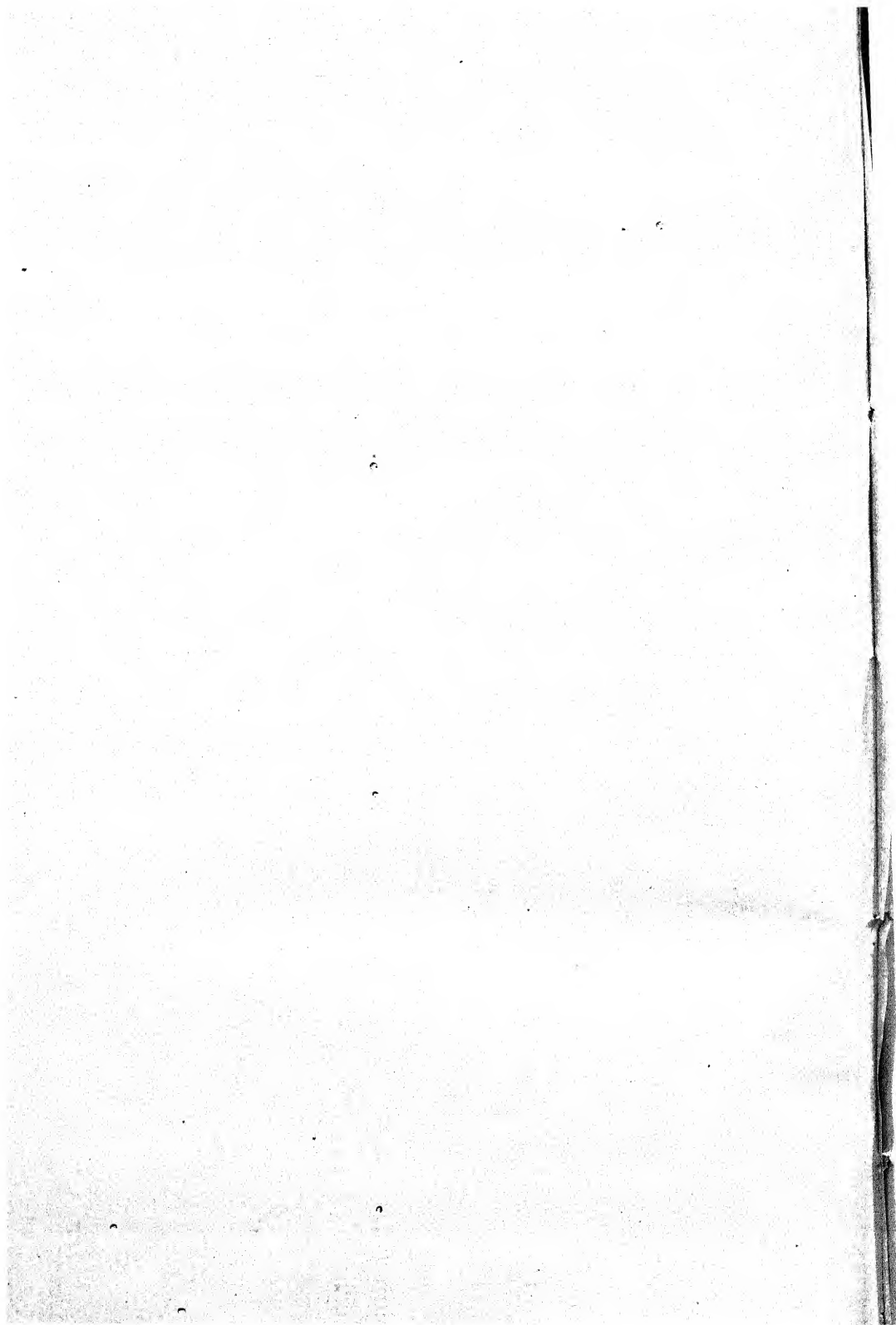
#### EXPLANATION OF CHARTS.

##### CHART I. (Class 2, Table I, p. 360.)

*Group I. Strains 1-3.* Strains 1 and 2 are *mono-ascospore* strains, and 3 is a *whole ascus* culture, from different perithecia on the same host. It will be seen that strain 1 shows aversion to 2 and 3, but 2 and 3 meet. All three cultures, although different, behave exactly the same towards all strains from other sources, except in Groups XII and XIII, when 2 and 3 behave in the same way towards the  $F_1$  of 2.

*Group II. Strains 5-17.* Mono-ascospore cultures obtained from several perithecia which developed in pure culture from a mass culture of ascospores isolated from Coe's Golden Drop plum. The whole group is of the same type; the strains show aversion to all the other strains except the  $F_1$  of strain 19. Strain 19 is included in Group III which proved to be the same as Group II, although isolated from two very different varieties of plum. Thus, where strains of the same type occur on different varieties of host they behave in the same way towards other strains from different sources, and the type of strain is irrespective of the variety of the host. A similar case was found in the mono-pycnospore cultures on such widely different hosts as apricot and Coe's Golden Drop plum.

*Group III. Strains 18-21.* Mono-ascospore strains isolated from mixed perithecia on bark of Prince of Wales plum. Strain 19 of this



group shows aversion to all other strains except its own  $F_1$  and Group II. All the other strains in this Group III behave in the same way.

*Group IV.* Mono-ascospore cultures from the same perithecium on a seedling plum New Orleans  $\times$  Victoria.

*Group V.* From the same host as Group IV, but on a different branch. These two groups, IV and V, show aversion to one another, although they occur on the same host.

*Groups VI, VII and VIII.* Isolated from three different perithecia on the stem of a young standard tree of seedling plum from Victoria selfed. One perithecium (Group VI) contains one type of strain and the other two groups are of another type, and yet all three behave the same towards all the groups from other sources.

*Groups IX, X, XI* are all  $F_1$  of strain 19 from three separate perithecia. They all behave alike, and show aversion to strain 2 and the  $F_1$  of strain 2, and no aversion *inter se* or to their own parent.

*Groups XII, XIII.*  $F_1$  of strain 2 from two separate perithecia. They also show aversion to 19 and the  $F_1$  of 19, but no aversion *inter se* or to their own parent.

#### CHART II. (Class 3, Table I, p. 360.)

*Group XIV.* Mono-ascospore strains 108-110, 116-133, and mono-ascus strains (111)-(115) all isolated from mixed perithecia which developed in mono-pycnospor culture  $SS_1$  (i.e. the  $F_1$  of  $SS_1$ ).

All the above cultures (108-133) show aversion towards the parent strain  $SS_1$ . On the  $F_1$  strains a few meet, but the majority show aversion *inter se*, and most of them show aversion towards the ten mono-pycnospor cultures with which they were tested.

*Group XV.* Mono-ascospore strains 134-197 are all from the same perithecium in mono-pycnospor culture  $SS_1$ . It will be seen that a few meet, but the majority show aversion *inter se*. The combinations were made somewhat at random, but in sufficient numbers to give some indication of the behaviour of the  $F_1$  strains towards one another.

#### CHART III. (Class 1, Table I, p. 360).

Shows the behaviour of the ten stock mono-pycnospor cultures used in Chart II, including  $SS_1$ . It will be noticed that there is one case of aversion in the same pycnidium, namely  $SS_4$ , which shows aversion towards  $SS_7$ .

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### *Discussion on heterothallism and sex in the Fungi.*

During recent years a considerable amount of work has been published dealing with heterothallism and sex in fungi. The work of Blakeslee (3-7) on the lower fungi is so well known that it need not be very fully discussed here, but it is interesting to note that in the lower fungi, as also in the Ustilaginales (15)—a lower group of the Basidiomycetes belonging to the higher fungi—sex is of a simple character, whereas in the Hymenomycetes—a group of the higher Basidiomycetes—it is of a complex nature.

Blakeslee could divide his *Mucors* into homo- and heterothallic forms; if heterothallic, the mycelia were either +, or -, or neutral, the latter being a combination of both + and -. If + and - strains are grown together the mycelia will fuse, two cells conjugate to form each zygospore, and a line of zygospores develops where the colonies meet. No zygospores will form if both colonies are of the same sex. The neutral strains will not combine with either + or -.

In the homothallic forms zygospores will develop on the same mycelium.

In the Ustilaginales or smuts two forms of spores are known, the intercalary chlamydospores and the sporidia or basidiospores. The sporidia conjugate in pairs, one nucleus passes over from one spore to the other, but the nuclei do not fuse. Germination then takes place, giving rise to a mycelium in the diplo-phase, with two nuclei in each cell. This condition persists until the development of the mature chlamydospore. The immature chlamydospore also contains two nuclei; these nuclei eventually fuse, so that the mature chlamydospore is mono-nucleate but diploid. The diploid chlamydospore germinates to form a structure called the promycelium (or basidium); the zygote nucleus of the developing promycelium divides twice, the first division being the reduction division, the second the homo-type division. One of the four daughter nuclei passes in each of the four sporidia given off from the promycelium. Kniep (15) working on a number of different forms of smuts has been able to show that in some forms, segregation into two sexes takes place at the reduction division. He does not appear to have isolated the four sporidia from a single promycelium, but the aggregate of a large number of isolations of single sporidia showed that only two sexes occurred and that in some cases, but not in all, the sexes were approximately equal in number. No neutral sporidia were found.

Sporidia are capable of budding and forming colonies when grown in pure culture on an artificial medium, somewhat after the manner of a

yeast, and all the secondary sporidia from a single sporidium proved to be of the same sex and would not conjugate, showing that segregation must have occurred before the development of the sporidia.

In the Hymenomycetes however sex is complicated by the fact that more than two types of sex can occur in one and the same fruiting body. In 1918 Mlle Bensaude (1), working on *Coprinus fimentarius*, was the first to show that heterothallism occurs in the Hymenomycetes, and the following year Kniep found heterothallism in *Schizophyllum commune* and a number of other Hymenomycetes.

In the heterothallic forms the mycelia fuse at an early stage; there is interchange of nuclei, giving rise to the diplo-phase with two nuclei (the dicaryons) in each cell. As in the Ustilaginales these nuclei do not fuse. Later clamp connections form on the young mycelium, short hooked processes which bend over so that their tips fuse with the hyphae from which they originate. Kniep (14) investigated the behaviour of these dicaryons in the region of the clamp connection. He observed that the nucleus nearest the apex of the hypha wanders into the clamp connection, the two nuclei divide conjugately and one half of each nucleus passes into the apical portion of the hyphae, to form the dicaryons of the newly formed cell. Thus the dicaryons which eventually pass into the young basidium are only distantly related to one another. In the basidium the dicaryons fuse, and the zygote nucleus then undergoes two divisions, as in the promycelium of the Ustilaginales.

Mlle Bensaude's criteria of sex in the Hymenomycetes are, the formation of clamp connections, the presence of dicaryons, conjugate division, and the formation of fruiting bodies. Kniep working independently observed clamp connections, dicaryons, and conjugate division in a number of other Hymenomycetes (12-14, 16, 17) but he also found that mono-sporous mycelia of heterothallic forms (12) were sometimes capable of producing mature fruiting bodies in the haploid condition without the intervention of any other strain. In such cases no clamp connections were produced, the cells were mono-nucleate and the spores were all of the same sex.

In homothallic forms clamp connections and dicaryons are always present, the outward and visible signs that the mycelium is in the diplo-phase.

Irene Mounce (19, 20) working on heterothallism in a number of species of the genus *Coprinus* came to the same conclusions as Kniep, namely, that heterothallism is common in the Hymenomycetes and that sex in this group is of a complex character, there being more than two

types. Her tables of combinations of different mono-sporous mycelia agree very closely with those published by Kniep. These conclusions were arrived at from the results of a number of mono-sporous mycelia from the same and from different fruiting bodies. It was not, however, until Kniep(17) was fortunate enough to find a Hymenomycete (*Aleurodiscus polygonius*) from which he could isolate the four basidiospores from a single basidium, that the nature of this complex form of sex could be determined. In *Aleurodiscus*, under cool conditions, each individual basidium sheds its four spores at the same time and the different basidia sporulate in succession, so that without much difficulty the four spores of a basidium can be isolated with certainty. In this fungus Kniep found that segregation into sexes took place at the first nuclear division of the diploid nucleus of the basidium. The diploid nucleus of the basidium divides twice, the first division being the reduction division, the second the homo-type division, giving rise to four nuclei. The basidium then forms four basidiospores, and one haploid nucleus passes into each spore. These four basidiospores germinate and form mycelia, two of which are of one sex and two of the other. He tested a large number of "Vierer-gruppen," or groups of four, from individual basidia, and invariably found that two and only two types of sex occurred in spores from the *same* basidium, and that the sexes

TABELLE I<sup>1</sup>.

	1	2	3	4
1	-	-	+	+
2	-	-	+	+
3	+	+	-	-
4	+	+	-	-

One "Vierer-gruppe."

segregated out in equal numbers (17) Tabelle I, p. 11. He then combined the four mycelia from *one* basidium with

(i) Mycelia from *other* basidia on the *same* fruiting body.

<sup>1</sup> In this, and the following Tables, the signs do not denote sex. The + sign indicates that fusion has taken place between haploid mycelia of different sexes, and the - sign indicates no fusion.



(ii) Mycelia from *different* fruiting bodies from the same spot and from different localities,

to see if the "Vierer-gruppen" were identical.

The results could be grouped under three headings.

A. From the *same* fruiting body.

(1) Fusion in pairs, in which case the four-groups are identical.

Tabelle I.

(2) No fusion at all.

B. From *different* fruiting bodies.

(3) Fusion of all mycelia however combined, or the reverse.

He then combined the "four-groups" from *different* basidia with 10 known strains from the *same* fruiting body, with the results shown in his table below.

TABELLE II (17, p. 11).

	1	2	3	4	5	6	7	8	9	10
3a	-	+	-	-	-	-	-	-	-	-
3b	-	+	-	-	-	-	-	-	-	-
3c	-	-	-	-	+	-	-	+	-	+
3d	-	-	-	-	+	-	-	+	-	+
5a	-	-	-	-	-	-	+	-	+	-
5b	-	-	-	-	-	-	+	-	+	-
5c	+	-	+	+	-	+	-	-	-	-
5d	+	-	+	+	-	+	-	-	-	-
10a	-	-	-	-	-	-	+	-	+	-
10b	-	-	-	-	-	-	+	-	+	-
10c	+	-	+	+	-	+	-	-	-	-
10d	+	-	+	+	-	+	-	-	-	-

Three "Vierer-gruppen" (i.e. 3, 5 and 10) combined with 10 known strains from the *same* fruiting body.

It can be seen at a glance that there are four distinct types of sex, and Kniep so far has always found four, and never more than four, types in the same fruiting body in *Aleurodiscus polygonius*.

Assuming that all the zygote nuclei in the basidia of the same fruiting body are identical, Kniep offers the following explanation.



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Since four different haplonts occur, then the basidia in the diploid phase cannot be homozygous, and sex must depend upon two pairs of factors,  $Aa$  and  $Bb$ . The zygote in the basidium will therefore be  $AaBb$ , and the four possible gametes or haplonts  $AB$ ,  $ab$ ,  $Ab$ ,  $aB$ . Out of 16 possible combinations, there are only nine genotypically different diplonts, four of which are homozygous in both factors, four homozygous in one factor, giving two different haplonts, and one heterozygous in both factors, giving the four different haplonts from which we started. As four different forms of sex mycelia are obtained from one fruiting body, only those combinations can occur in which the resulting zygote is heterozygous in both factors (selective fertilisation). The genes in one basidium segregate out to form spores of the constitution  $AB$  and  $ab$  (Tabelle III) and in another basidium  $Ab$ ,  $aB$  (Tabelle IV). Kniep found that these two types of basidia occurred in approximately equal numbers. Each of these types should be capable of fusion of mycelia *inter se*, but not with the other type, for the haplonts of these basidia cannot form diplonts heterozygous in both factors, and therefore, as Kniep's results showed, no fusion can occur between them.

TABELLE III.

		1	2	3	4
		$AB$	$AB$	$ab$	$ab$
1	$AB$	—	—	+	+
2	$AB$	—	—	+	+
3	$ab$	+	+	—	—
4	$ab$	+	+	—	—

Basidium I.

TABELLE IV.

		1	2	3	4
		$Ab$	$Ab$	$aB$	$aB$
1	$Ab$	—	—	+	+
2	$Ab$	—	—	+	+
3	$aB$	+	+	—	—
4	$aB$	+	+	—	—

Basidium II.

Different basidia from the same fruiting body.

To explain the fusion of all mycelia from *different* fruiting bodies (B 3, p. 367), Kniep suggests multiple allelomorphism, but he points out that he has no definite proof of this in *Aleurodiscus*, as, so far, he has not been able to obtain the hybrid  $F_1$  fruiting body ( $F_1$  sporophyte) from combinations of haplonts from different fruiting bodies. His experiments with *Schizophyllum commune* populations, however, have

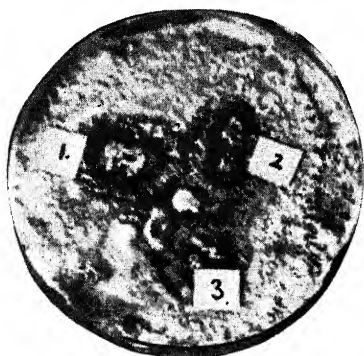


Fig. 4.  $2 + F_1$  of  $2 + F_1$  of 2.

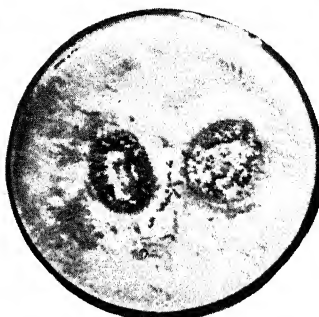


Fig. 1.  $19 + 19$ .

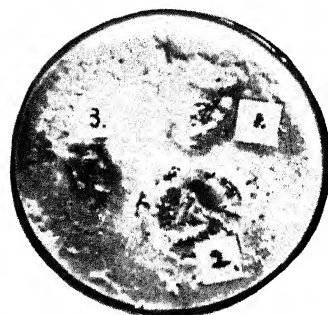


Fig. 7.  $19 + F_1$  of  $19 + F_1$  of 19.

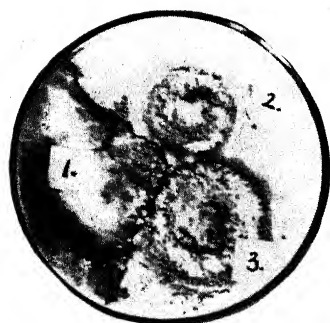


Fig. 5.  $F_1$  of  $2 + F_1$  of  $19 + F_1$  of 19.

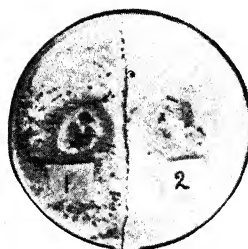


Fig. 2.  $2 + 9$ .



Fig. 8.  $19 + F_1$  of  $19 + F_1$  of 2.

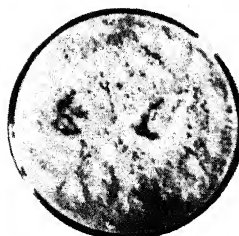
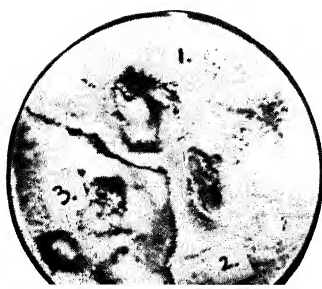
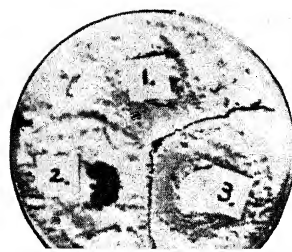
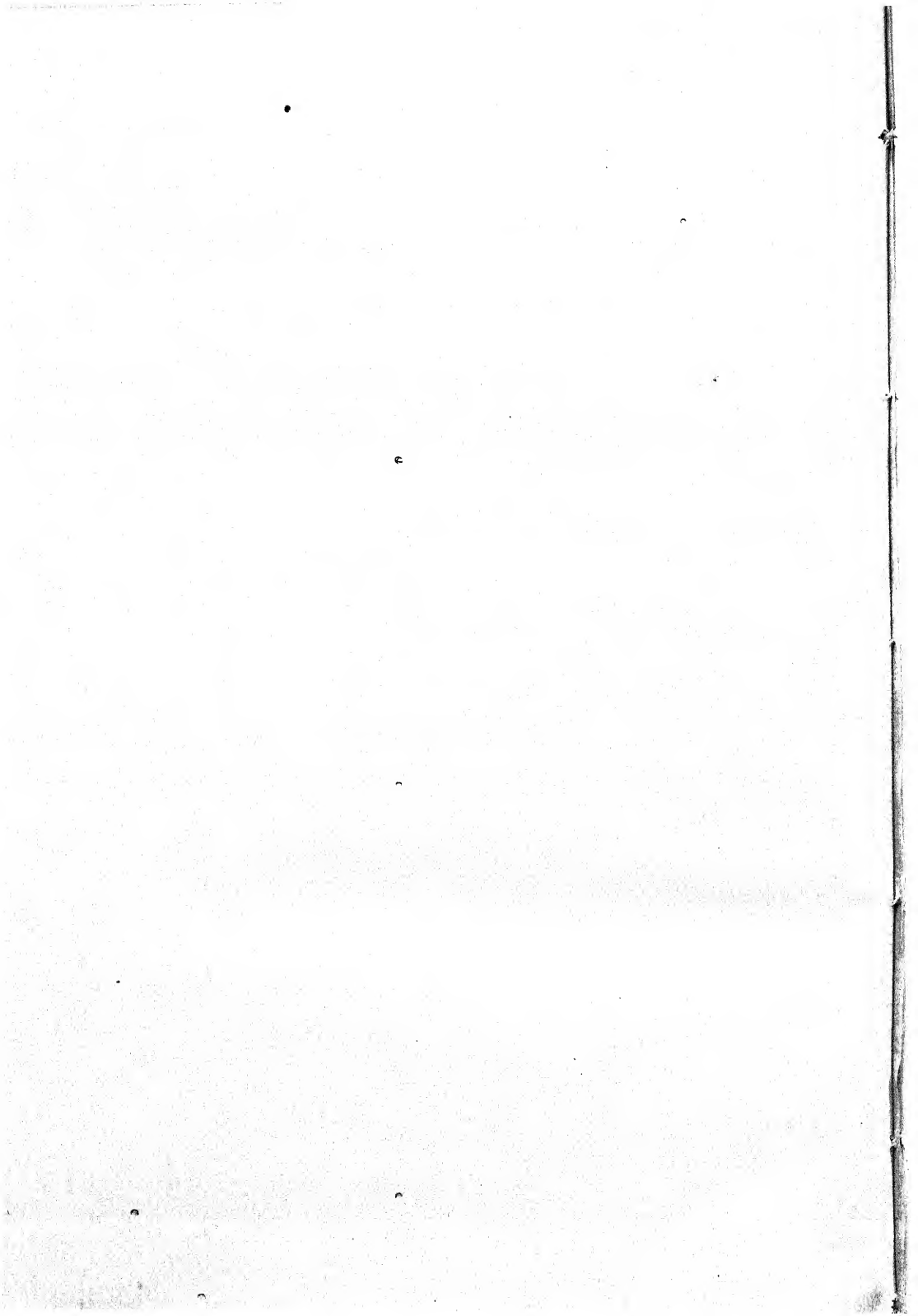


Fig. 3.  $2 + 2$ .





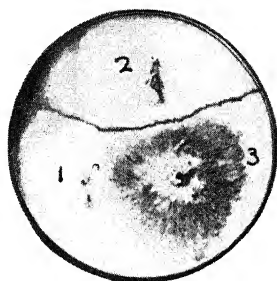


Fig. 1.

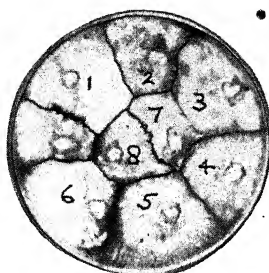


Fig. 5.

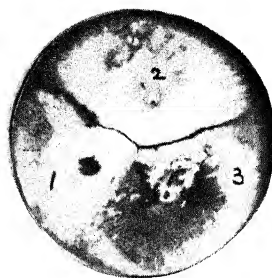


Fig. 2.

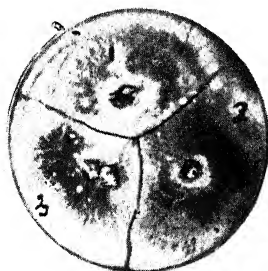


Fig. 3.

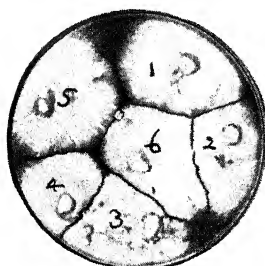


Fig. 6.

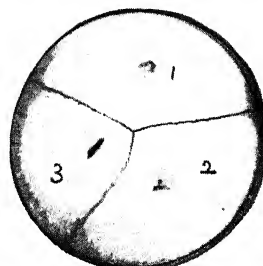


Fig. 4.

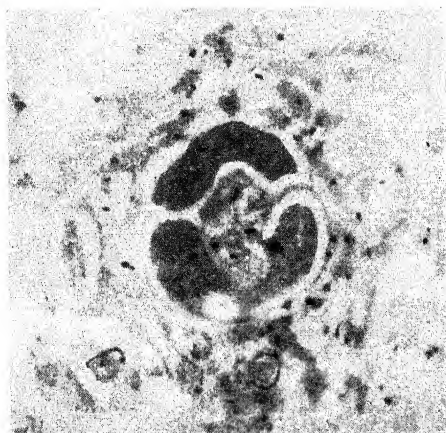


Fig. 7.

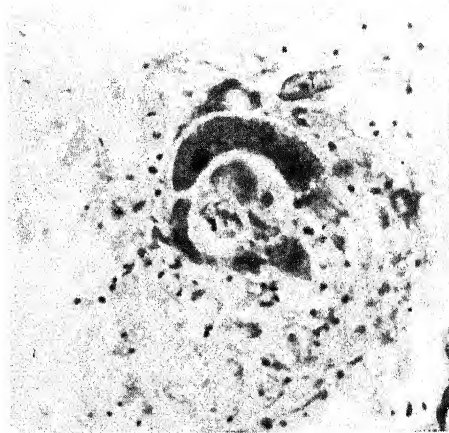


Fig. 8.





led him to adopt this explanation. In this fungus he raised a number of hybrid  $F_1$  sporophytes and from these he isolated mono-spore mycelia ( $F_1$ -gametophytes) and found (in some cases but not in all) four types of sex in the haploid  $F_1$ -generation.

In conclusion I wish to express my thanks to Prof. H. Kniep for his courtesy in allowing me to reproduce some of his Tables.

## EXPLANATION OF PLATES.

### PLATE XIX.

*Mono-ascospore cultures included in Class 2. (Table I, p. 360.)*

- Fig. 1. Culture  $SA_{19}$  combined with itself. (Parent strain.) *No aversion.*
- Fig. 2. Culture  $SA_{19}$  combined with  $SA_2$ . *Aversion.*
- Fig. 3. Culture  $SA_2$  combined with itself. (Parent strain.) *No aversion.*
- Fig. 4. Culture  $SA_2$  combined with two colonies of its  $F_1$ . *No aversion.*
- Fig. 5.  $F_1$  of  $SA_2$  (col. 1) combined with two colonies of the  $F_1$  of  $SA_{19}$  (colonies 2 and 3). *Aversion.*
- Fig. 6. Two colonies of the  $F_1$  of  $SA_2$  (cols. 1 and 2) combined with one colony of  $F_1$  of  $SA_{19}$ . *Aversion.*
- Fig. 7. Culture  $SA_{19}$  (col. 1) combined with two colonies of its own  $F_1$  (cols. 2 and 3). *No aversion.*
- Fig. 8. Culture  $SA_{19}$  (col. 1) combined with one colony of its own  $F_1$  (col. 2) and one colony of the  $F_1$  of  $SA_2$  (col. 3). *Aversion.*
- Fig. 9. Culture  $SA_{19}$  (col. 1) combined with one colony of its own  $\bar{F}_1$  (col. 2) and one colony of the  $F_1$  of  $SA_2$  (col. 3). *Aversion.*

### PLATE XX.

*Mono-ascospore cultures included in Class 3. (Table I, p. 360.)*

- Fig. 1. Two  $F_1$  mono-ascospore mycelia of  $SS_1$  (cols. 2 and 3) combined with stock mono-pycnosspore  $SS_{10}$  (see Charts II and III).  $SS_{10}$  meets col. 3 but shows *aversion* to col. 2.
- Fig. 2. Three  $F_1$  mono-ascospore mycelia of  $SS_1$ . Cols. 2 and 3 are from the same perithegium, col. 1 from another perithegium.
- Fig. 3. Three  $F_1$  mono-ascospore mycelia of  $SS_1$  from the same perithegium. *General aversion.*
- Fig. 4. Two  $F_1$  mono-ascospore mycelia of  $SS_1$  (cols. 2 and 3) combined with the parent mono-pycnosspore culture (col. 1). *General aversion.*
- Fig. 5. Eight mono-ascospore mycelia of  $SS_1$  from the same perithegium. *General aversion.*
- Fig. 6. Six mono-ascospore mycelia of  $SS_1$  } *aversion.*
- Figs. 7 and 8. Two consecutive sections through a young perithegium in  $SS_1$  on oat-agar, showing archicarp.
- Fig. 9. Section through line of demarcation between two averting strains on oat-agar.
  - (a) aerial mycelium.
  - (b) submerged mycelium.
  - (c) crack in the agar.

The micro-photographs on Plate II were taken by Mr Osterstock, and the rest of the photographs by A. F. Emarton, laboratory assistant in this Institution.



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# ON THE OFFSPRING OF RABBIT-DOES MATED WITH TWO SIRES SIMULTANEOUSLY.

BY STEFAN KOPEĆ.

(Government Institute for Agricultural Research, Putawy, Poland.)

(With One Text-figure.)

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## INTRODUCTION.

FROM various observations on mammals, the impregnation of several eggs of one female by two or more males during the same rutting time (superfecundatio) seems to be unquestionable. The question arises whether the fetuses derived from different sires have any influence on each other, or whether they are mutually independent, when simultaneously developing in one female.

This question, being rather a problem of physiology than of genetics, cannot be *a priori* solved by means of the genetic independence ascribed to each foetus. (Cf. general discussion.) Certain chance observations made in this direction by husbandmen, as well as analogical descriptions by zoologists as v. Rath ('95-'98), Tornier ('96 and '98), and others, are not based on adequate comparative materials, nor on exact study of the characters discussed. These observations speak, it is true, in favour of the supposition that the heterogeneous fetuses develop totally independently from each other, but being based on genetically unanalysed materials they cannot be considered as convincing. The same reservation



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must be made with regard to the ingenious experiments of Heape ('90 and '98) who grafted fertilized eggs from a doe belonging to one breed into the uterus of a female of another strain, and arrived at results pointing to totally independent development of the various foetuses.

### MATERIAL AND METHODS.

My experiments have been performed on Himalayan rabbits bred in my laboratory for the last four years, and thoroughly examined in regard to pureness of their breed, as well as to variability of various characters. The does used in the experiments were first of all mated with one and the same Himalayan buck, in order to establish the hue and the weight of pure bred Himalayan offspring from these females. After some litters of Himalayan rabbits had been obtained, the females were mated with one and the same Silver buck derived from the author's own well examined pure Silver breed. Not until the uniform type of the resulting hybrids had been ascertained were the Himalayan does used for the experiment proper, i.e. they were mated with the formerly used Himalayan sire and with the Silver male simultaneously during one rutting-period. Of the 24 double matings only 3 produced offspring by two sires. The females which were fecundated by two different sires were mated in the following way:

1. Female No. 8 was mated by a Himalayan and by a Silver sire, one immediately after the other, at 5.44 in the morning; by a Himalayan sire at 2.20 p.m. and by a Silver buck at 2.55 p.m.

2. Female No. 11 was mated by a Silver and by a Himalayan male, one immediately after the other, at 11.25 a.m., as well as at 11.50 a.m.

3. Female No. 25 was mated like female No. 11 at 11.45 a.m. and at 6.35 p.m.

In the remaining 21 experiments (which failed) the does mated by two different sires produced offspring derived exclusively from one of the two males. The origin of the new-born rabbits could undoubtedly be ascertained by their totally different hue.

The weight of the rabbits was determined by weighing the new-borns before their first suckling, which does not as a rule take place here at once after birth. Fragments of grams were considered as full grams. The specimens which had suckled could easily be distinguished by their abdomina filled with milk and they were excluded from the comparative study of weight of new-born rabbits. (Cf. analogous observations made by King, '15, on albino rats.)

The weights of separate new-borns are recorded in Table I. The average weights of all new-borns of each material studied are given in Table II. The standard deviation ( $\sigma$ ) was calculated from the special formula for scanty material,  $\sigma = \pm \sqrt{\frac{\sum (pa^2)}{n-1}}$ ,  $a$  being the difference between the weights of separate variates and the average,  $p$  the number of the variates, and  $n$  the total number of the specimens under examination. Since the author united data referring to the progeny from all females belonging to one material, it ought to be emphasized that the

TABLE I.

*Weights of Separate New-borns in Grams.*

A			B			C			D			E		F	G				H	
Offspring of Himalayan Female×Himalayan Male			Offspring of Himalayan Female×Silver Male			Himalayan offspring of Himalayan Female mated with Himalayan Male and Silver Male simultaneously			Hybrids produced by Himalayan Female mated with Himalayan Male and Silver Male simultaneously			Offspring of specimens from material A		Offspring of specimens from material C	Offspring of specimens from material B				Offspring of specimens from material D	
♀8	♀11	♀25	♀8	♀11	♀25	♀8	♀11	♀25	♀8	♀11	♀25	♀8a	♀36	♀85	♀69	♀51	♀89	♀86	♀87	
21	29	28	34	32	38	31	33	36	44	39	50	40	40	32	23	20	18	21	20	
27	29	29	34	33	38	33	35	39	49	41	51	42	42	36	26	26	35	27	22	
28	29	31	37	35	38	34	40	43	—	45	—	44	43	36	31	31	41	34	34	
29	31	31	38	36	40	39	—	—	—	—	—	44	44	37	32	35	41	37	35	
30	31	32	38	36	41	—	—	—	—	—	—	45	45	38	33	36	42	38	37	
30	32	34	39	37	42	—	—	—	—	—	—	46	46	39	33	37	42	39	37	
30	33	34	39	39	43	—	—	—	—	—	—	—	—	40	33	37	43	40	42	
30	34	35	40	40	45	—	—	—	—	—	—	—	—	42	33	38	44	42	42	
30	34	36	40	42	47	—	—	—	—	—	—	—	—	42	33	38	45	43	43	
30	35	36	40	—	48	—	—	—	—	—	—	—	—	43	34	39	46	43	43	
31	—	37	41	—	48	—	—	—	—	—	—	—	—	44	36	40	46	43	45	
31	—	37	41	—	49	—	—	—	—	—	—	—	—	44	40	40	47	46	45	
31	—	38	42	—	—	—	—	—	—	—	—	—	—	45	41	40	47	46	45	
32	—	38	42	—	—	—	—	—	—	—	—	—	—	48	41	42	49	47	46	
33	—	39	42	—	—	—	—	—	—	—	—	—	—	48	41	42	49	52	47	
34	—	39	43	—	—	—	—	—	—	—	—	—	—	48	42	42	49	52	49	
34	—	40	44	—	—	—	—	—	—	—	—	—	—	48	42	43	50	53	49	
36	—	40	46	—	—	—	—	—	—	—	—	—	—	50	42	44	50	53	51	
38	—	40	46	—	—	—	—	—	—	—	—	—	—	—	42	44	51	53	55	
39	—	41	47	—	—	—	—	—	—	—	—	—	—	—	43	45	52	54	57	
39	—	42	47	—	—	—	—	—	—	—	—	—	—	—	43	45	55	57	58	
40	—	42	48	—	—	—	—	—	—	—	—	—	—	—	44	45	56	59	62	
—	—	42	—	—	—	—	—	—	—	—	—	—	—	—	46	46	56	—	—	
—	—	43	—	—	—	—	—	—	—	—	—	—	—	—	46	47	58	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	46	48	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	47	49	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	48	50	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	48	54	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	49	57	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	50	60	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	50	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	52	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	52	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	52	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	52	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	58	—	—	—	—	

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TABLE II.

*Average Weights of the New-borns in Grams.*

Materials	Data referring to the offspring of each female of the material examined				Data referring to the offspring of all females of the material examined			
	Number corresponding to each female	Average litter-size	Average weight	Number of specimens	Average litter-size	Average weight with the corresponding probable error	$\pm \sigma$	Number of specimens
Offspring of Himalayan ♀ × Himalayan ♂	8	5.5	31.95	22	5.0	34.00 ± 0.43	4.74	56
	11	5.0	31.70	10				
	25	4.8	36.83	24				
Offspring of Himalayan ♀ × Silver ♂	8	4.4	41.27	22	4.3	40.81 ± 0.46	4.44	43
	11	4.5	36.67	9				
	25	4.0	43.08	12				
Himalayan offspring of Himalayan ♀ mated with Himalayan ♂ and Silver ♂ simultaneously	8	6.0	34.25	4	5.7	36.30 ± 0.81	3.80	10
	11	6.0	36.00	3				
	25	5.0	39.33	3				
Hybrids produced by Himalayan ♀ mated with malayan ♂ and Silver ♂ simultaneously	8	6.0	46.50	2	5.7	45.57 ± 1.18	4.61	7
	11	6.0	41.66	3				
	25	5.0	50.50	2				
Offspring of specimens in material A	8	3.0	43.50	6	3.0	43.41 ± 0.40	2.07	12
	36	3.0	43.33	6				
Offspring of specimens in material C	85	3.0	42.22	18	3.0	42.22 ± 0.82	5.15	18
	69	5.0	41.49	35				
Offspring of specimens in material B	51	6.0	42.00	30	5.3	42.97 ± 0.59	8.32	89
	89	4.8	46.33	24				
	86	5.5	44.50	22				
Offspring of specimens in material D	87	5.5	43.82	22	5.5	44.16 ± 1.01	9.94	44

Himalayan does were sisters of the same age. All females were identically fed during the periods of gestation as well as beyond these periods, all other conditions, viz. light, humidity, temperature, being the same for each female. As the weight of separate specimens of a lot in rabbits is in inverse proportion to their number, it must be noticed that in materials A—D and G—H only such litters were taken into consideration as contained the most frequent numbers of new-borns, i.e. from 4 to 6 specimens. (Cf. Hammond, '21 and Kopeć, '23.) Materials E and F of Himalayan rabbits consisted exclusively of litters containing only 3 specimens. Consequently the weights of separate new-borns and their averages are larger here than in the remaining cases, and ought not to be directly compared therewith.

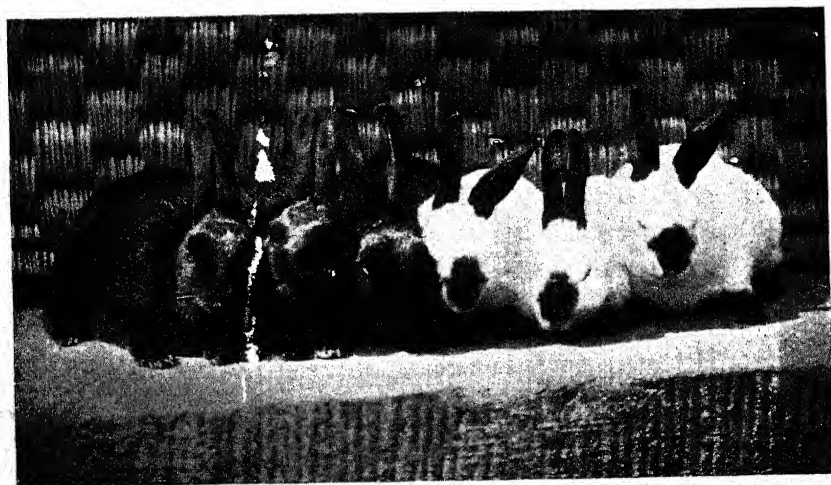
## DESCRIPTION OF THE OFFSPRING.

As has been stated above, the origin of the new-borns could be ascertained by the colour of their skin. In one of the successful experiments (female No. 8), 4 Himalayan rabbits and 2 hybrids were obtained,

in the second (female No. 11), 3 Himalayans and 3 hybrids, in the third (female No. 25), 3 Himalayans and 2 hybrids.

*2. The Colour.*

Litters derived from heterogeneous impregnation consisted of rabbits having the typical Himalayan pattern and of specimens whose uniform colour did in no way differ from the characteristic colour of Himalayan — Silver hybrids. (Cf. photo. in text.) Not only the colour of skin, pink



Full-grown offspring from Himalayan doe No. 11 mated with a Himalayan and a Silver buck during one rutting time. On the right three pure Himalayans by the Himalayan sire, on the left three hybrids characteristic of  $F_1$  of the cross between Himalayan doe and Silver buck.

in new-born Himalayans and greyish-blue in the hybrids, and the final hue of full-grown rabbits, but even the mode of its development and the rate of these processes were the same in specimens derived from normal matings and in those from heterogeneous fecundation. The same must be said as to the colour of the eyes. The above macroscopical statement was also ascertained by exact histological research. Nor did the first filial generation of such rabbits from double mating give evidence of any changes of their respective pattern.

B. *The Weight*<sup>1</sup>.

On comparing the weight of pure Himalayan new-borns with the analogous weights of normal hybrids from Himalayan does by a Silver buck we see that, owing to the larger size of the Silver strain to which the father belonged, the weight of hybrids is larger than that of normal Himalayan specimens. (Cf. Tables, materials *A* and *B*.) The difference between the respective weights calculated from the data of Table II amounts to  $6.81 \pm 0.63$  gs, and, being 10.8 times larger than its probable error, it ought to be considered as essential. From the comparison of the average weights of Himalayan new-borns with the average weights of hybrids, both derived from heterogeneous impregnation (materials *C* and *D*), it follows that the weight of hybrids is larger by  $9.27 \pm 1.43$  gs than that of Himalayan new-borns. The difference being 6.5 times larger than the probable error, the weight of hybrids from heterogeneous fecundation is also essentially larger than that of the Himalayans. We may therefore infer that when an essential difference does exist between two breeds, it may also manifest itself when these two breeds of rabbits are developing simultaneously.

In spite of the maintenance of these differences of weight, the heterogeneous new-borns show certain changes which prove that the presence of Himalayan fetuses has a positive influence on the weight of hybrids and *vice versa*, i.e. that the development of the two classes of fetuses in cases of successful double mating is not entirely independent, in so far as weight is concerned. Thus, the weight of normal Himalayan new-borns is from 21 to 43 gs, while that of the Himalayans from heterogeneous fecundation is higher, oscillating from 31 to 43 gs. (Cf. Table I.) From Table II we see that the average weight of the normal Himalayan new-borns from female No. 8 was 31.95, from female No. 11—31.70, from female No. 25—36.83 gs. The average weight of Himalayan new-borns from double mating from the same females was always higher, viz. it amounted to 34.25, 36.00 and 39.33 gs respectively. The difference of the averages of these two Himalayan materials calculated from the data of Table II amounts to  $2.30 \pm 0.92$  gs. We see that the difference is only 2.5 times larger than its probable error and it may therefore not

<sup>1</sup> This study is based on material differing in two respects from that described in the formerly published Polish paper (*Mém. Inst. Nat. Polonais d'Économie Rurale à Putawy*, Vol. 2, 1922), viz. 1, the number of the new-borns examined is considerably greater in certain materials; 2, the offspring of females Nos. 71, 91 and 99 are excluded, as these does were soon used for other experiments. Owing to these changes also the appertaining biometrical constants concerning the weight of new-borns were, partly at least, changed.

be considered as essential. In respect to the above it ought to be emphasized that the number of new-borns in one litter of common Himalayan matings was 5.0, while litters from heterogeneous fecundation contained on the average 5.7 or nearly 6.0 specimens. As was stated above, the weight of the new-born rabbits is in inverse relation to the number of specimens in one litter. It follows that in our calculations the weight of the Himalayan new-borns from double mating is too low in comparison with the examined normal Himalayan specimens. After having equalized the number of new-borns in one litter in both materials we should obtain a difference between the weight of Himalayan offspring of normal and of double matings which would undoubtedly be considerably larger than that above recorded.

The increased weight of the new-borns derived from double fecundation in comparison with those from normal mating becomes still more evident if we compare the weight of new-born hybrids. As may be inferred from our Tables of data (material *B*) the weight of normal hybrids oscillates in the limits from 32 to 49 gs, while that of the new-borns from heterogeneous fecundation amounted to from 39 to 51 gs (material *D*). The difference of the averages was here  $4.76 \pm 1.27$  gs, and being 3.7 times larger than its probable error it ought to be stated that the weight of new-born hybrids from double matings is essentially larger than that of normal litters.

The weight of the new-born rabbits does not undergo any essential changes depending on the age of the females, at least during the first two years of their life during which these females have been examined. (Kopeć, '23.) It may consequently be ascertained that the presence in one female of heterogeneous foetuses deriving from double mating may have an essential positive influence on the weight of the new-borns of both breeds. The material being here rather scanty, it ought to be noticed that the above recorded differences of the average weights of the two kinds of new-borns have been ascertained in all the three females mated with two different sires simultaneously. This concordance points to a general validity of the results obtained here.

On examining the offspring of the enlarged specimens from double mating it is found that the weight of the first filial generation is normal. (Cf. Tables, materials *E* and *F*, as well as *G* and *H*.) The difference of weight of the offspring of normal Himalayan new-borns and of those from heterogeneous fecundation calculated from the data of Table II is  $1.19 \pm 0.91$  gs. The corresponding difference of the offspring in hybrids of the two kinds is  $-1.19 \pm 1.17$  gs. The differences being here once positive



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and once negative, and approximatively equal with their probable error, we need not consider further the positive influence on the weight of new-borns of simultaneously developing foetuses by two sires in the first generation.

#### GENERAL DISCUSSION.

By the above described experiments it has been for the first time methodically proved that the two breeds of new-borns deriving from successful double mating of rabbit-does by two different bucks may be totally independent in regard to their respective hue.

From my observations it follows moreover that the difference of weight of the rabbits of the two breeds persists in spite of their simultaneous development in one female. This result is in agreement with the present views on the heredity of body weight. But, notwithstanding the maintenance of such differences in our experiments, foetuses of one father affect in a favourable manner the weight of those of the other, when simultaneously developing in one mother. Consequently, the foetuses of the two origins do not develop in total independence from each other in respect to their weight. The question arises as to the cause of the essential positive change of body-weight, i.e. of a genetically determined character.

In order to solve this problem we must take into consideration the mutual interchange of substances, which, according to present views, exists between mother and foetus<sup>1</sup>, viz. not only various food-stuffs or salts, alkaloids, narcotics, organic acids, which were artificially introduced into the body, but even such compound bodies as antitoxins and most probably also such specific substances having the characters of hormones, as iodothyrene, passing through the placenta from the mother to the embryo. On the other hand, numerous observations are known to us which point to the fact that various substances pass from the embryo to the mother. The well-known albumen reaction of Abderhalden, which points to the presence of foreign substances in the blood of pregnant females, seems not as yet to be sufficiently confirmed, but experiments on injections of extracts from foetuses, or from the foetal parts of the placenta, into females, show that substances are produced by the foetus or by the foetal part of the placenta, which pass into the blood of the mother and cause the secretion of milk, aches, blood-coagulation, changes of blood-pressure, etc. It ought also to be noticed that parts of the villi of the foetal placenta forming ingrowths into the blood-vessels of

<sup>1</sup> As to detailed references, cf. Biedl ('16), Wolff ('13) and Zuntz ('08).